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TITLE PAGE

Title

Poliprotect vs Omeprazole in the relief of heartburn, epigastric pain and burning in patients without erosive esophagitis and gastro-duodenal lesions: A Randomized, Controlled Trial

Short title

RCT comparing a MPA vs PPI in Heartburn and EPS

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Abbreviations
AE, Adverse Event; EPS, Epigastric Pain Syndrome; FD, Functional Dyspepsia; FH, Functional Heartburn; GIQLI, Gastrointestinal Quality of Life Index; GSRS, Gastrointestinal Symptom Rating Scale; IP, Investigational Product; ITT, Intention To Treat; MPA, Mucosal Protective Agent; NERD, Non-Erosive Reflux Disease; OTE, Overall Treatment Evaluation; PP, Per Protocol; PPI, Proton Pump Inhibitor; RCT, Randomized Clinical Trial; RH, Reflux Hypersensitivity; VAS, Visual Analogue Scale

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ABSTRACT

Background. In the treatment of upper GI endoscopy-negative patients with heartburn and epigastric pain or burning, antacids, anti-reflux agents, and mucosal protective agents (MPA) are widely used, alone or as add-on treatment to increase response to proton pump inhibitors, which are not indicated in infancy and pregnancy, and account for significant cost expenditure.

Aims & Methods: In this randomized, controlled, double-blind, double-dummy, multicenter trial assessing the efficacy and safety of MPA Poliprotect (neoBianacid®) versus Omeprazole in the relief of heartburn and epigastric pain/burning, 275 endoscopy-negative outpatients were given a 4-week treatment with Omeprazole (20 mg q.d.) or Poliprotect (5 times a day for the initial 2 weeks, and on-demand thereafter), followed by an open-label 4-week treatment period with Poliprotect on demand. Gut microbiota change was assessed.

Results: A 2-week treatment with Poliprotect proved non-inferior to Omeprazole for symptom relief (between-group difference in the change in VAS symptom score: [mean, 95% CI] -5.4, -9.9 to -0.1; -6.2, -10.8 to -1.6; ITT and PP populations, respectively).

Poliprotect's benefit remained unaltered after shifting to on-demand intake, with no gut microbiota variation. The initial benefit of Omeprazole was maintained against significantly higher use of rescue medicine sachets (mean, 95% CI: Poliprotect 3.9, 2.8–5.0; Omeprazole 8.2, 4.8–11.6) and associated with an increased abundance of oral cavity genera in the intestinal microbiota. No relevant adverse events were reported in either treatment arm.

Conclusions: Poliprotect proved non-inferior to standard-dose Omeprazole in symptomatic patients with heartburn/epigastric burning without erosive esophagitis and gastro-duodenal lesions. Gut microbiota was not affected by Poliprotect treatment. The study is registered in Clinicaltrial.gov (NCT03238534) and the EudraCT Database (2015-005216-15).

- **Keywords:** Dyspepsia**;** Epigastric Pain Syndrome; Gut Microbiota; Medical Device Made of Natural Substances; Mucosal Protective Agent; Non-Erosive Reflux Disease; Omeprazole.

Introduction

Heartburn and the Epigastric Pain Syndrome (EPS) subtype of Functional Dyspepsia (FD) are highly prevalent symptomatic conditions in endoscopy-negative patients that impair patients' quality of life (1). As stated, based on questionnaires and history taking alone, it can be clinically impossible to distinguish the two conditions (2, 3); according to sound evidence (4, 5), proton pump inhibitors (PPIs) are the recommended treatment of choice (2, 6-8) for both conditions, even though they may interact with co-administered drugs (9), are not indicated in infancy and pregnancy, and account for significant cost expenditure (10).

Mucosal protective agents (MPA) adhere to the gastroesophageal epithelium, reinforce the mucosal barrier, and protect the epithelium from acid and non-acid luminal components. MPAs improve gastroesophageal reflux (11) and FD symptoms (12) when added to a standard PPI treatment. So far, no controlled studies have assessed the benefit of MPA as monotherapy for heartburn and epigastric pain/burning in endoscopy-negative patients. In animal and *ex vivo* human studies, MPA Poliprotect significantly decreased ethanol- and indomethacin-induced gastric mucosa lesions and damage to esophageal mucosal integrity induced by acid-pepsin-bile solution, as assessed by transepithelial electrical resistance and the ulcerogenic index,

respectively. It also maintained 36% mucoadhesivity for at least 2 hours, and counteracted the oxidative stress induced by 2,2'-Azobis(2-amidinopropane) dihydrochloride, *in vitro* (13) and unpublished proprietary data from the product's technical dossier). Post-marketing surveillance, consisting of large-scale, validated observational surveys, confirmed its effectiveness in relieving heartburn and/or epigastric pain or burning and did not report any serious AEs (14).

The aim of the study was to compare the clinical efficacy and safety of Poliprotect with standard-dose Omeprazole in the treatment of endoscopy-negative patients with heartburn and/or epigastric pain or burning. As an exploratory aim, changes in the gut microbiota after Poliprotect and PPI treatment were assessed.

Materials and Methods

Study design

This randomized, controlled, double-blind, double-dummy, non-inferiority trial, carried out in thirteen Italian hospitals, aimed to evaluate the efficacy and safety of Poliprotect (neoBianacid®, 1.55 g, Aboca, Sansepolcro, Italy) versus PPI (Omeprazole 20 mg, Doc Generici Srl, Milano, Italy) in the relief of heartburn and/or epigastric pain or burning. The study design is reported in the Supplemental Digital Content (Supplemental Digital Figure S1). The product, a CE-marked medical device marketed in 15 European countries, is a 100% natural product composed of Poliprotect (a polysaccharide fraction from *Aloe vera, Malva sylvestris* and *Althea officinalis;* minerals limestone and nahcolite) and a flavonoidic fraction from *Glycyrrhiza glabra* and *Matricaria recutita*).

Study population

Male and female patients aged 18–70 years (inclusive) were selected.

All patients were interviewed at the first visit with an identical printed questionnaire containing the key questions to detect the presence, the frequency, and the onset of symptoms matching the Montreal definition of heartburn and of the Rome III EPS.

The main inclusion criteria were: symptoms of heartburn and/or epigastric pain/burning (Epigastric Pain Syndrome, EPS; Rome III criteria) (15); a negative upper endoscopy, to be performed during the screening period if not performed in the last three years; a VAS score ≥30 mm and ≤70 mm (VAS related to heartburn/epigastric pain or burning) for at least 6 of the 14 days preceding the screening visit; willingness not to make diet and lifestyle changes during the trial.

The main exclusion criteria were: esophagitis (Los Angeles A–D) or Barrett's esophagus; active gastric or duodenal ulcer; previous gastric or major GI surgery; heartburn/epigastric pain/burning that has not previously responded to antacid or PPI treatment; current intake of any drugs that could affect symptoms; clinically significant disease of any body system; pregnant, breastfeeding or fertile women without contraception.

Detailed and comprehensive selection criteria are reported in the Supplemental Digital Content (Supplemental Table S1).

The study protocol, approved by the competent authorities and the ethics committees of each participating center, was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and all relevant regulations. Written Informed Consent was obtained from all participants.

Blinding

Before randomizing a patient, the investigator or designee had to contact the centralized treatment allocation system and provide some information (such as patient number, date of birth, and date of visit). In order to keep the patients blind to the randomized treatment, a double-dummy method was used. Blinding, packaging, and distribution of investigational kits were managed by a qualified contract research organization. The Poliprotect and its placebo were supplied in hydrolytic class III amber glass bottles, sealed with aluminum caps. The PPI and its placebo were supplied in high-density polyethylene bottles, sealed with child-proof closures. All of the participants in the study (i.e., patients, investigators, research nurses, the study coordinator, personnel involved in monitoring study, data entry personnel) were blinded to the identity of the study treatment and had no access to the patient codes.

Procedures

After a 2-week screening/wash-out period (V-1 to V0), subjects who met the selection criteria were randomized, ensuring a 1:1 ratio, into either the PPI group (PPI verum + Poliprotect placebo) or the Poliprotect group (PPI placebo + Poliprotect verum) for a double-blind 4-week period (V0-V2). During this randomized, double-blind 4-week period, the Poliprotect treatment schedule was different in the first 2 weeks (V0-V1) compared to the following 2 weeks (V1-V2). In the first two weeks, one tablet of Poliprotect had to be taken five times a day (30 min after breakfast, lunch and dinner; midafternoon; before going to bed), whereas in the following two weeks, Poliprotect intake was on demand (up to eight times/day), defined as the product intake necessary to reach the healthy state of V1. Throughout the double-blind period, the PPI had to be taken once a day, 30 minutes before breakfast. For the remaining 4-week period (V2-V3), the blinding was removed, and all patients were administered Poliprotect *verum* only, on demand. Magaldrate oral gel (Riopan Gel, 80 mg/ml, Takeda Italia S.p.A.) was allowed as rescue medication if needed (one sachet at a time, at any time of day) throughout the study treatment periods. Patients were recommended to record their VAS symptom score, concomitant medications, investigational products (IPs) and rescue therapy intake in once-daily paper diaries in the evenings. The Gastrointestinal Quality of Life Index (GIQLI), Gastrointestinal Symptom Rating Scale (GSRS), and Overall Treatment Evaluation (OTE; starting from V1) questionnaires were administered at each visit (16-18).

Outcomes

The primary efficacy endpoint was the comparison between the two groups in terms of the severity of heartburn and/or epigastric pain or burning from baseline to V1, as measured by means of a 100-mm VAS (from “no symptoms” to “overwhelming symptoms”) reported in the patients’ daily diaries.

Secondary efficacy endpoints were the comparison between groups of the VAS score for the severity of heartburn and/or epigastric pain or burning at day 1, day 3, and day 7; comparison between groups of the VAS score for the severity of heartburn and/or epigastric pain or burning from week 2 onwards; the quantity, number of days of use, and proportion of patients using rescue medication; the on-demand intake of Poliprotect; the change in GIQLI and GSRS score at each visit versus baseline; and OTE scores at each visit.

The results of the safety assessment, except for laboratory test results, are shown in the Supplemental Digital Content (Supplemental Digital Table S5).

Data assessment and Statistical analysis

Sample sizes of 110 per group achieve 85% power to detect non-inferiority (one-sided, two-sample t-test; significance level 0.025), using a non-inferiority margin of -11 for the difference between groups in the absolute change in mean VAS score from baseline to day 13, assuming an expected standard deviation of 27 for the change in VAS score in the standard therapy group (19).

According to the approved protocol, where the non-inferiority margin is defined as a percentage of the standard mean (i.e., the mean change previously reported for the standard therapy group) and the alternative hypothesis is that the means for the two treatments differ by no more than 25% of the standard mean, the value of -11 was pre-established as the non-inferiority margin, corresponding to 25% of the mean change in VAS score (i.e., 44 units) observed in a similar population after a two-week course of PPI treatment, with the same PPI, dose and dosage (19). A non-inferiority margin of -11 was considered clinically acceptable by the investigators and was determined according to the technical recommendation (20) that the test treatment should retain at least a certain and clinically relevant amount of the previously-shown superiority of the active comparator over placebo (21-23).

With an estimated 25% of patients not being evaluable for the primary endpoint for any reason, the total sample size was 276 patients. The intention-to-treat (ITT) population included all randomized patients who received at least one dose of the IP and was identical to the safety analysis population. The per-protocol (PP) population included all ITT subjects who completed the double-blind treatment period without major protocol deviations. The primary efficacy analysis was performed in both the ITT and PP populations. Secondary efficacy analyses were conducted in the ITT population. The methods of microbiota analysis used on the available fecal samples at both V0 and V2 are detailed in the Supplemental Digital Content (Supplemental Digital Methods).

In analyzing the VAS score as a continuous variable, the average score for the 5 days prior to the two consecutive reference visits has been considered, with no imputation of missing values. For early time points (day 1, 3, and 7), the VAS score values for the day immediately before were used in cases of missing data. In determining the response rate (categorical variable), responder patients were defined as those patients with at least a 50% improvement in VAS symptoms. Patients with a missing VAS score in each of the 5 days prior to any of the two reference visits

were considered non-responders. Patients with missing questionnaire data were excluded from the analysis of the corresponding period.

The OTE score, which rates symptom change on a 15-point scale (-7 to -1 = worse; 0 = no change; +1 to +7 = better), was recoded as negative if scores were in the “worse” or “no change” range, and otherwise as positive, as previously reported (18).

Compliance with treatments was calculated as the ratio of the amount of IP taken (from the daily diary record) to what was expected to be taken according to the treatment schedule.

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Between October 15, 2017, and September 03, 2021, 373 patients were screened and 98 did not meet the inclusion criteria. Thus, 275 patients were enrolled and randomly assigned to treatments. Of these, 257 were treated and 17 were drop-outs (Figure 1). A summary of the patients’ demographic and clinical characteristics and a list of screening failures are shown in Table 1 and in the Supplemental Digital Content (Supplemental Digital Table S7), respectively. Eighteen randomized people did not take even one treatment dose: the Covid pandemic and related environmental difficulties contributed to this decision. Compliance was equal to or greater than 90% in both treatment groups (Supplemental Digital Table S3).

In both analyses, the lower limit of the two-sided 95% CI for the difference between groups in the change in VAS symptom score from V0 to V1 lies above the pre-established non-inferiority margin of -11 (-5.4, 95% CI -9.9 to -0.1; -6.2, 95% CI -10.8 to -1.6; ITT and PP populations, respectively) (Figure 2B).

Mean VAS scores were not significantly different between groups at baseline or at any subsequent time points in the study [V0, 45.1 (42.7 – 47.5) and 47.2 (44.7 – 49.8), P = 0.31; V1, 30.2 (26.9 – 33.5) and 26.4 (22.7 – 30.0), P = 0.12; V2, 25.7 (22.4 – 28.8) and 25.4 (21.7–29.2), P = 0.94; V3, 21.7 (18.3 – 25.2) and 24.2 (20.4 – 28), P = 0.34; mean (95% CI), Poliprotect and PPI group, respectively] (Figure 2A and Supplemental Digital Table S2). From V1 to V2, VAS score decreased more in the Poliprotect group (-3.89) than in the PPI group (-0.76) (Figure 2A), albeit the difference was not significant (-3.13, 95% CI -6.74 to 0.47; P = 0.09), even after excluding patients taking rescue medication (P = 0.06) (data not shown).

Likewise, the proportions of responders were not significantly different between groups [V1: 46/131 (35.1%) and 56/126 (44.4%), P = 0.16; V2: 60/128 (46.8%) and 56/121 (46.2%), P = 1; V3: 70/124 (56.4%) and 61/116 (52.5%), P = 0.64; Poliprotect and PPI group, respectively].

The number of rescue medication sachets used was significantly lower in the Poliprotect group than the Omeprazole group in the V1-V2 (P = 0.019) and V2-V3 (P = 0.032) periods (Figure 2C and Supplemental Digital Table S4), despite no difference in VAS score and comparable on-demand intake of Poliprotect during both V1-V2 [tablets/day: 2.11 (1.90) and 2.23 (2.05); mean (SD), Poliprotect and PPI group, respectively; P = 0.604] and V2-V3 [tablets/day: 2.11 (1.97) and 2.36 (2.14); mean (SD), Poliprotect and PPI group, respectively; P = 0.543] (Figure 2D), even when comparing subgroups of patients who had taken and patients who had not taken rescue medication (data not shown).

The number of days of use of rescue medication (data not shown) was significantly lower in the Poliprotect group in the V1-V2 period (P = 0.013) and remained lower in the V2-V3 period, although not significantly (P = 0.156).

There were no significant differences between groups in VAS score 1, 3, and 7 days after treatment start (Supplemental Digital Table S2). All GSRS and GIQLI domain scores showed a trend of progressive improvement from V0 to V3 in both treatment groups, except for the GSRS reflux domain in the Omeprazole group (Supplemental Digital Figure S2). Domain score changes did not differ between groups at any time points, except for a significant difference favoring the Omeprazole group for the GSRS reflux syndrome domain at V1 (P = 0.017), the constipation syndrome domain at V1 and V3 (P = 0.05 and P = 0.03, respectively), and the GIQLI gastrointestinal symptom domain at V1 (P = 0.004) (Supplemental Digital Table S2).

The proportion of Omeprazole-treated patients reporting a positive OTE score decreased in the V1-V2 period to stabilize thereafter, whereas it remained stable in the V1-V2 period to increase thereafter in the Poliprotect group (Supplemental Digital Table S2 and Figure S2).

A total of 178 AEs were recorded, with 79 (37.4%) in the Poliprotect arm and 99 (38.1%) in the PPI arm, none of which was serious; 4 of them, all occurring in the PPI group, were considered as related to the IP (Supplemental Digital Table S5). None of the assessed laboratory test items was altered or significantly different between groups (data not shown).

Microbiota results

No significant within-group changes over time or between-group differences or partitions at both V0 and V2 were evidenced by alpha or beta diversity analysis, respectively (Figure 3). As compared to Poliprotect, a significantly higher degree of variability from V0 to V2 was found in the PPI group with respect to the Bray-Curtis dissimilarity distance: in particular, a significant enrichment of the species *Streptococcus salivarius* and *Streptococcus sinensis* and a significant increase over time in the relative abundance of *Haemophilus parainfluenzae*, *Streptococcus dentisani*, *Streptococcus parasanguinis*, and *Veillonella dispar* ($p<0.0001$ for each) were observed in the PPI group (Figure 3 and Supplemental Digital Figure S4).

Post-hoc analyses

In a post-hoc analysis performed on the two sub-populations of patients presenting with heartburn only (N=177) and those with epigastric pain/burning with or without heartburn (N=80), no significant differences were found between the subgroups in terms of baseline characteristics, including symptom severity score (Table 1), or post-baseline mean VAS score (Supplemental Digital Table S6). Likewise, the proportions of responders, defined as those patients with at least 50% symptom improvement according to the VAS, were not significantly different at any visit, comparing the subgroups of patients presenting with heartburn only and those with epigastric pain/burning (Supplemental Digital Table S6).

Discussion

A 2-week treatment with Poliprotect tablets, five times daily, provides non-inferior efficacy compared to Omeprazole 20 mg once daily for the relief of heartburn and epigastric pain or burning in adult symptomatic patients without erosive esophagitis and gastro-duodenal lesions, likely including a mix of non-erosive reflux disease, functional esophageal disorders and functional dyspepsia. To our knowledge, only two Randomized Clinical Trials (RCTs) have reported that add-on treatment with irsogladine- and hyaluronic acid-based MPAs significantly enhanced the positive effect of PPIs in patients with NERD (non-erosive reflux disease) (11, 24). No RCT has compared the efficacy of an MPA versus a PPI in the treatment of heartburn and epigastric pain or burning.

Heartburn, epigastric pain, and epigastric burning are three slightly different and often interchangeably subjective sensory representations of pain. The general clinical use of these symptoms to identify and differentiate patients with reflux-like symptoms from the dyspeptic EPS subtype is not supported by several studies (25-27) or by a recent international expert consensus (2). In this RCT, all but 17 of the 80 EPS patients complained of both epigastric pain/burning and heartburn and did not significantly differ from the patients presenting only heartburn in terms of baseline characteristics, including VAS score, post-baseline mean VAS score or response rate (VAS), further confirming that EPS patients are not clinically distinguishable from endoscopy-negative patients with heartburn (26-29). Patients-reported outcome (PRO) instruments like RDQ and GERDQ are useful to capture GERD symptoms, but not epigastric pain and burning as well. There are no validated Italian PRO instruments for dyspeptic symptoms. The Italian GSRS instrument has not been validated for test-retest reliability, and its internal consistency is unsatisfactory for the abdominal pain domain and barely satisfactory for the reflux domain (30). VAS score is a well standardized tool for assessing pain, and it has been widely used to assess painful upper GI symptoms. We have thus used VAS for the primary pain endpoint and GSRS for the secondary ones, capturing the GI symptoms that often accompany reflux and dyspeptic symptoms.

The clinical benefit of Poliprotect and Omeprazole, as assessed by VAS score, is evident as early as the first day of treatment and increases over the 2-week period. Thereafter, the favorable effect of Poliprotect continues to increase, albeit slightly, even after switching from 5 times daily to on-demand intake, with a lesser mean daily consumption (on average 2-3 tablets/day). This effect was not affected by rescue medicine use, which was relatively stable. It would therefore appear that the initial benefit of Poliprotect can be maintained with on-demand treatment at lower daily consumption for at least 6 weeks. It would seem that, consistent with previous experience with PPIs in NERD (31, 32), the initial benefit obtained with daily Poliprotect in the treatment of symptomatic endoscopy-negative patients with heartburn and epigastric pain/burning can be maintained with on-demand therapy.

Based on the above-mentioned preclinical studies, the clinical benefit of MPA relies on providing the mucosa with a complex, mucus-like, adherent, antioxidant, pH-buffering matrix, thus limiting the stimulation of acid, bile and other luminal sensitizers on the gastroesophageal epithelium. The Rome III criteria for EPS were used in the protocol, which was approved before the publication of Rome IV. Nonetheless, as the term “bothersome” is the only addition to the Rome III criteria for

EPS in Rome IV, and as we included only patients with a VAS score >30, the patients in this trial met the Rome IV criteria for EPS. The results of this trial can be reasonably extrapolated to the general population with moderate symptoms of heartburn and/or epigastric pain/burning, since the patients were recruited in open-access outpatient clinics and, in addition, many of them were referred by general practitioners who had previously been instructed to send the trial centers any unselected patients presenting with heartburn and/or epigastric pain/burning. As patients reporting an initial VAS score >70 were not included, the effect of Poliprotect appears to benefit patients with moderate symptom severity, but this cannot be extrapolated to patients with high symptom severity. Unlike with Poliprotect, the initial benefit of Omeprazole is maintained against significantly higher usage of rescue medicine compared to the Poliprotect arm and despite daily Omeprazole intake, confirming that PPIs cannot effectively control even moderate symptoms in a subgroup of endoscopy-negative patients with heartburn and EPS patients (33-35).

Several reasons may account for the greater demand for rescue therapy in at least some of the patients on PPIs: sensitization of the esophagus to weakly acidic reflux by preceding acid exposure and/or due to impaired epithelial integrity was supported by studies on persistent symptoms despite PPIs; also, partial gastric acid suppression may have still allowed a few symptomatic acid refluxes to occur (36-38). An esophageal pH impedance test would have been helpful for clarifying this; however, this invasive diagnostic investigation is rarely performed in general practice, and it would have hindered the recruitment rate in the outpatient clinic population.

Limitation of this trial is that a specific GERD phenotype for which Poliprotect is optimal cannot be inferred from this study. Instead, this is potentially an option for the symptomatic foregut patient without erosive esophagitis and gastro-duodenal lesions in whom a PPI would typically be considered.

An esophageal pH impedance test would have been helpful for clarifying whether the treatment response might have been related to the presence of gastroesophageal reflux, which frequently occurs in EPS patients and in endoscopy-negative patients with heartburn. According to the outcome of a pH impedance test, endoscopy-negative patients may present with abnormal acid or non-acid reflux (NERD), symptomatic normal acid or non-acid reflux (Reflux Hypersensitivity, RH), or normal asymptomatic reflux (Functional Heartburn, FH). Increased gastroesophageal acid reflux is associated with the presence of epigastric pain in dyspeptic heartburn-negative patients (26) and is detected in up to 50% of functional dyspepsia patients complaining of epigastric burning without predominant typical reflux symptoms (27). It is thus conceivable that patients with acid reflux benefited from PPI treatment. Likewise, in the absence of a pH impedance test, we cannot say which patients benefited most from Poliprotect treatment: those with or those without gastroesophageal reflux. Nonetheless, we can argue that patients with either acid or non-acid gastroesophageal reflux might have benefited from the combined effect of epithelial barrier protection, antacids, and the antioxidant properties of Poliprotect. Such interpretation might explain the significantly lesser use of rescue medicine in the Poliprotect group than in the Omeprazole group. In any case, only further properly-designed RCTs can verify the comparative efficacy of Poliprotect and PPIs in patients with endoscopy-negative gastroesophageal reflux disease-like symptoms according to phenotype based on esophageal pH impedance monitoring.

Heartburn and epigastric pain/burning often overlap with other dyspeptic and intestinal symptoms, and the GSRS questionnaire confirmed this association. It is notable that the improvement in heartburn and epigastric pain/burning obtained with PPI and Poliprotect was accompanied by a parallel improvement in the associated dyspeptic and intestinal symptoms. This finding confirms a previous observation in GERD patients with overlapping dyspepsia and IBS-like symptoms treated with PPI (39), and also shows that the improvement in heartburn and epigastric pain/burning obtained with Poliprotect is associated with a parallel improvement in the accompanying dyspeptic and intestinal symptoms during six weeks of on-demand treatment with 2-3 tablets/day.

During the last unblinded 4 weeks of the study, the two groups did not differ in terms of Poliprotect consumption and symptomatic benefit, which, in the Omeprazole arm, was obtained alongside a significant increase in antacid rescue medicine as compared to the Poliprotect arm. This finding is in line with the notion that PPI suspension can be followed by symptoms worsening due to the gastric acid rebound effect (40).

The 4-week Omeprazole treatment is comparable with most previous controlled trials with PPIs in NERD and FD patients, since prolonging the treatment does not add symptomatic benefits (5, 41).

Within the above-mentioned limits of the Italian GSRS questionnaire, Omeprazole performed better than Poliprotect in the reflux and constipation GSRS domains as well as in the gastrointestinal symptom GIGLI domain in the first two weeks of treatment, and not differently in the following two weeks. It would therefore appear that PPI also have an initial and temporary favorable effect on regurgitation that, unlike with the VAS, is captured by the reflux domain. The favorable and temporary effect of PPI on the constipation domain can be interpreted as the result of a possible increase in intestinal fermentation and/or in absorption alterations, both of which affect stool consistency and intestinal transit and have been related to the hyposecretory effects of PPIs and the consequent increase in bacterial colonization in the small bowel (42, 43).

Helicobacter pylori status was not assessed, since the symptomatic response to PPI is not affected by *H. pylori* in these patients, while the modest positive response to *H. pylori* eradication in FD patients is only apparent after months (44, 45).

The study did not assess patients for hiatal hernia, NERD, RH and FH subtypes, eosinophilic esophagitis or prior antacid or PPI use. Patients not previously responding to PPI and antacids were excluded from the trial in order to limit the inclusion of patients in whom symptoms are not caused by reflux or caused by non-acid reflux, who might have biased the study against PPI or Poliprotect (which acts also as an antacid). Nonetheless, since we did not exclude PPI and antacid naive patients, it was still possible that some patients not responding to PPI and some patients not responding to “antacid” Poliprotect were included in the trial. However, several considerations make unlikely that this may have given the Poliprotect treatment, or Omeprazole treatment as well, an unfair advantage. Firstly, given the high number of patients randomized to one or the other of the 2 treatments, randomization guarantees that the likely small number of PPI and antacid naive patients in this sample were equally distributed between the two treatment arms. Moreover, this study and previously reported data do not support the presence of a

randomization bias, affecting the comparative outcome of this trial. Indeed, (a) PPI treated patients performed better than the Poliprotect treated ones at V1, both as assessed by VAS symptom score and GSRS reflux domain score; (b) the 48% proportion of responders in the subgroup with heartburn only (Supplemental Digital Table S6), after 4 weeks of PPI treatment is comparable to the expected 49% response rate to PPI reported in a meta-analysis of 12 studies in patients with heartburn in the setting of normal endoscopy (46). In addition, since we did not exclude also antacid naïve patients, the observed symptomatic benefit of Poliprotect could have been limited by the presence of patients not responding to antacids.

In the PPI arm, the initial benefit of the treatment was progressively lost in the following two weeks, as evidenced by a parallel increase in rescue medicine use and a decrease in positive OTE by more than 10% from V1 to V2 (Supplemental Digital Figure S2 and Table S2). The trend in improvement throughout the treatment period was continuously progressive in the Poliprotect arm for all efficacy variables, whereas it was not maintained in the Omeprazole arm for the GSRS reflux domain, the GIQLI emotional dysfunction domain, or the positive OTE score.

We confirmed (47, 48) that PPI treatment is associated with an increased abundance of oral cavity genera in the intestinal microbiota. Significant changes of microbiome composition after 4 weeks of 40 mg omeprazole, twice daily, in healthy volunteers have been previously reported (49). Our data show for the first time that a microbiota change can take place after 4 weeks even with the 20 mg daily dose of omeprazole in a patient population.

Unlike the hyposecretive action of PPIs, the buffering activity of Poliprotect, exerted by the bicarbonate minerals embedded in the complex vegetable matrix adhered to the epithelial lining, does not affect the amount of intraluminal acid secretion and hence the microbiota composition.

In conclusion, starting from the first day of treatment, Poliprotect proved non-inferior to Omeprazole in the relief of heartburn, epigastric pain and burning in the initial two weeks, and even better on demand (on average 2-3 tablets/day) compared to Omeprazole in the subsequent two weeks. In addition, Poliprotect on demand counteracted the predictable worsening of symptoms that follows the suspension of PPI treatment. Furthermore, the MPA is a 100% natural product and therefore biodegradable by definition, with no impact on the environment (50), and in the present RCT, it showed high safety without affecting the gut microbiota.

In large-scale surveys, aimed at the post-marketing surveillance of Poliprotect, 3,471 physicians and 848 patients did not report any serious AEs. In addition, physicians largely reported good tolerability in the Poliprotect-treated population, which included pregnant women and children (14). Post-market vigilance data reported an incidence rate <1/10.000 of non-serious gastrointestinal and skin adverse effects of Poliprotect and no serious adverse effects. Based on the results of this trial and considering such a high safety level, it is conceivable that Poliprotect might be used as first line treatment for heartburn and EPS, and to substitute PPI in those conditions in which they are contraindicated. However, further RCTs are required to assess whether Poliprotect has the potential to be indicated on a cost-benefit and cost-effective basis for the treatment of heartburn and epigastric pain and burning in endoscopy-negative patients as an alternative to PPIs.

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Figure Legends

Figure 1. Patient distribution.

Figure 2. Mean absolute symptom score values, results of primary analysis and use of rescue medication and Poliprotect on demand. (A) Mean absolute values of symptom severity score, as measured by means of a 100-mm visual analog scale (VAS; from “no symptoms” equal to 0 mm to “overwhelming symptoms” equal to 100 mm). From V2 to V3, depicted with a colorful background, the comparison treatment (PPI) and blinding were both removed, and all patients were administered Poliprotect verum only, on demand. The I bars represent standard error. (B) Difference in absolute change in VAS symptom severity score from baseline to day 13 (V0–V1) between the Omeprazole group and the Poliprotect group and relative 95% confidence interval, supporting the hypothesis that Poliprotect was non-inferior to Omeprazole. In both the per-protocol and intention-to-treat populations, the inferiority hypothesis is rejected by means of the unilateral unpaired Student’s t-test shifted by -11 (minus the non-inferiority threshold) on the change in symptom score between baseline and Visit 1 (p= 0.020 and p= 0008 for non-inferiority, respectively). (C) Number of sachets of rescue medication used during the indicated study periods. (D) Number of tablets of Poliprotect (verum or placebo) used on demand. * $p<0.05$

Figure 3. Analysis of microbiome. a) Color-coded box plots showing α-diversity estimators, measured for each group at different time points. b) PCoA plot of bacterial β-diversity based on Bray-Curtis dissimilarity and weighted UniFrac distance according to individual health status. For each group, the 95% confidence interval has been drawn. Numbers between parentheses represent the percentage of the total variance explained by the principal coordinates. c) Box plots showing the distribution of differences in the inter-individual distances over time (V2-V0) for both considered beta measures. d) Color-coded box plots showing the distribution of bacterial species that were significantly enriched at V2 with respect to V0. A p-value ≤ 0.05 was considered statistically significant.

Table 1. Demographic and Baseline Characteristics of the intention-to-treat population.

	Poliprotect			PPI		
	All (n= 131)	Heartburn (n= 96)	Epigastric Pain* (n= 35)	All (n=126)	Heartburn (n= 81)	Epigastric Pain* (n= 45)
Age (y), mean (SD)	48.9 ± 11.9	48.8 ± 11.7	49.3 ± 12.7	47.7 ± 12.6	47.4 ± 12.6	48.1 ± 12.8
Sex, n (%)						
Female	81 (61.8%)	61 (63.5%)	20 (57.1%)	81 (64.3%)	51 (63.0%)	30 (66.7%)
Male	50 (38.2%)	35 (36.5%)	15 (42.9)	45 (35.7%)	30 (37%)	15 (33.3%)
Race, n (%)						
Caucasian	130 (99.2%)	95 (99%)	35 (100%)	123 (97.6%)	79 (97.5%)	44 (97.8%)
Asian	0 (0%)	0	0 (0%)	1 (0.4%)	0 (0.0%)	1 (2.2%)
Hispanic	1 (0.8%)	1	0 (0%)	1 (0.8%)	1 (1.2%)	0 (0%)
African/American	0 (0%)	0	0 (0%)	1 (0.8%)	1 (1.2%)	0 (0%)
BMI (kg/m^2), mean (SD)	25 ± 3.6	24.7 ± 3.4	25.7 ± 4.1	24.1 ± 4	23.7 ± 3.5	24.8 ± 4.6
VAS score (mm), mean (95% CI)	45.6 (43.3-48.0)	46.6 (44.1-49.0)	43.0 (37.3-48.7)	47.4 (44.9- 49.9)	49.0 (46.0-52.1)	44.4 (40.0-48.9)
GSRS Questionnaire, mean (95% CI)						
Abdominal pain domain	1.0 (0.9-1.1)	1.0 (0.9-1.1)	1.1 (0.9-1.3)	1.1 (1.0-1.2)	1.0 (0.9-1.2)	1.3 (1.1-1.4)
Reflux syndrome domain	1.4 (1.3-1.5)	1.5 (1.3-1.6)	1.3 (1.1-1.6)	1.4 (1.3-1.6)	1.4 (1.3-1.6)	1.4 (1.2-1.6)
Diarrhea syndrome domain	0.5 (0.4-0.6)	0.5 (0.4-0.6)	0.5 (0.4-0.7)	0.5 (0.4-0.6)	0.6 (0.4-0.7)	0.4 (0.2-0.5)
Indigestion syndrome domain	1.2 (1.1-1.2)	1.1 (1.0-1.2)	1.2 (1.0-1.3)	1.2 (1.1-1.3)	1.1 (1.0-1.2)	1.3 (1.2-1.5)
Constipation syndrome domain	0.6 (0.5-0.7)	0.6 (0.5-0.7)	0.7 (0.5-0.9)	0.7 (0.6-0.8)	0.7 (0.5-0.8)	0.7 (0.5-0.9)
GIQLI Questionnaire, mean (95% CI)						
Gastrointestinal symptoms domain	48.9 (47.2-50.5)	48.9 (47.0-50.8)	48.7 (45.3-52.0)	47.0 (45.5-48.6)	47.9 (45.8-49.9)	45.5 (43.2-47.9)
Emotional dysfunction	11.2 (10.6-11.9)	11.3 (10.6-12.1)	11.0 (9.7-12.3)	10.7 (10.1-11.4)	11.1 (10.2-11.9)	10.1 (9.0-11.3)
Physical dysfunction	16.9 (16.0-17.8)	17.2 (16.2-18.2)	16.1 (14.2-18.0)	16.4 (15.5-17.2)	16.6 (15.5-17.7)	15.9 (14.3-17.5)
Social dysfunction	12.8 (12.2-13.3)	12.9 (12.3-13.4)	12.5 (11.3-13.7)	12.6 (12.1-13.1)	12.7 (12.0-13.3)	12.6 (11.8-13.3)
Treatment effects	3.3 (3.1-3.5)	3.3 (3.1-3.5)	3.3 (3.0-3.7)	3.4 (3.2-3.5)	3.4 (3.2-3.6)	3.2 (2.9-3.6)
Total score	93.1 (89.9-96.2)	93.6 (90.0-97.2)	91.6 (84.8-98.3)	90.2 (87.1-93.2)	91.7 (87.8-95.5)	87.4 (82.5-92.3)

Baseline characteristics were similar in the two treatment arms, even if clinical subgroups were compared. *Patients presenting epigastric pain/burning with or without heartburn. BMI= Body Mass Index, VAS= Visual Analog Scale, PPI= proton pump inhibitor, CI= Confidence Interval