

# Randomised clinical trial: mucosal protection combined with acid suppression in the treatment of non-erosive reflux disease – efficacy of Esoxx, a hyaluronic acid–chondroitin sulphate based bioadhesive formulation

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## SUMMARY

### Background

Several studies have shown that patients with non-erosive reflux disease (NERD) are less responsive to proton pump inhibitors (PPIs) than those with erosive disease as they belong to different subgroups, in whom factors other than acid can trigger symptoms.

### Aim

To evaluate whether combined therapy (mucosal protection plus acid suppression) would improve symptom relief compared to PPI treatment alone.

### Methods

In a multicenter, randomised, double-blind trial, 154 patients with NERD were randomised to receive Esoxx (Alfa Wassermann, Bologna, Italy), a hyaluronic acid-chondroitin sulphate based bioadhesive formulation, or placebo, in addition to acid suppression with standard dose PPIs for 2 weeks. Symptoms (heartburn, acid regurgitation, retrosternal pain and acid taste in the mouth) and health-related quality of life (HRQL) were evaluated before and after treatment. The primary endpoint was the proportion of patients with at least a 3-point reduction in the total symptom score.

### Results

At the end of treatment, the primary endpoint was reached by 52.6% of patients taking Esoxx compared to 32.1% of those given placebo ( $P < 0.01$ ). The same was true also for HRQL, evaluated by means of the Short Form-36 questionnaire, which improved with both treatments, but some items were significantly better after Esoxx plus PPI therapy.

### Conclusion

The synergistic effect of Esoxx with PPI treatment suggests that mucosal protection added to acid suppression could improve symptoms and HRQL in NERD patients.

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## INTRODUCTION

Gastro-oesophageal reflux disease (GERD) is a highly prevalent disorder in Western countries, as its predominant symptom, heartburn, can occur once a week in up to 26% of the general population.<sup>1</sup> Despite geographical variations, the prevalence of GERD is increasing worldwide.

Over the past decade, it has been realised that there are two different phenotypes of the disease. Some patients present oesophageal mucosal lesions (i.e. erosive oesophagitis), but the majority (up to 70%) have a macroscopically normal mucosa at endoscopy. Such patients are usually considered to have non-erosive reflux disease (NERD).<sup>2–4</sup>

Proton pump inhibitors (PPIs) represent the first choice medical treatment for GERD,<sup>5</sup> in that they are able to provide an 80–85% healing rate for oesophageal lesions, including ulcers, and also reduce the incidence of complications. Pooled analyses<sup>6, 7</sup> have shown that in 56–76% of cases, symptom relief can also be achieved, even though this benefit seems to be reduced in patients with NERD. According to a widely quoted systematic review,<sup>7</sup> compared to patients with erosive oesophagitis, patients with NERD display a reduced symptom relief with PPIs, with about 20% reduction of therapeutic gain. A large AGA survey<sup>8</sup> found that – despite PPI use – over 55% of subjects with GERD symptoms in the general population (where non-erosive and erosive diseases are obviously mixed) report continued disruption of their quality of life.

Recent investigations have shown that not only acidic, but also non-acidic refluxes are able to induce the histopathological alterations, which have been clearly documented by electron and light microscopy in the majority of NERD patients.<sup>9–11</sup> In particular, the dilation of intercellular spaces between adjacent cells of the oesophageal epithelium represents a feature that has become the hallmark of microscopic oesophagitis. This intercellular gap leads to increased permeability that favours the penetration of hydrogen ions and other substances (including pepsin and bile) into oesophageal sub-mucosa, thus reaching nerve fibres, whose stimulation generates the typical symptom heartburn. Several studies<sup>12</sup> suggested a synergistic action between acid and duodeno-gastric reflux in inducing lesions. The important role of pepsin in the pathogenesis of extra-oesophageal manifestations of GERD is increasingly being appreciated.<sup>13</sup>

An ideal therapy for NERD patients should – in addition to acid secretion – address all the above-mentioned pathophysiologic features, that is provide a barrier to

(and/or bind) the residual aggressive components of the refluxate (i.e. weakly acidic content and pepsin) while stimulating mucosal repair. To achieve these goals, a class III medical device, Esoxx (Alfa Wassermann, Bologna, Italy), was specifically designed and developed.<sup>14, 15</sup> It consists of a mixture (1:2.5 ratio) of low molecular weight (80–100 kDa) hyaluronic acid and low molecular weight (10–20 kDa) chondroitin sulphate, dispersed in a bioadhesive carrier (poloxamer 407) to form a macromolecular complex, coating the oesophageal mucosa and acting as a mechanical barrier against the noxious components of the refluxate. Transit time of liquids through the oesophagus is very short (less than 16 s), even in a supine subject.<sup>16</sup> A viscous liquid formulation that adheres to and coat the mucosa will limit the contact of refluxed acid and pepsin with the epithelial surface<sup>17</sup> and can act as a vehicle to deliver drugs for local action within the oesophagus.<sup>18</sup>

The components of Esoxx are two well-known physiologic substances. Hyaluronic acid is a widespread, biologically active substance, which regulates cellular function through interaction with specific receptors.<sup>19</sup> It is a multifunctional, high molecular weight glycosaminoglycan, component of the majority of extracellular matrices and involved in several key physiologic processes, including wound repair and regeneration, morphogenesis and matrix organisation.<sup>20</sup> The biological roles of hyaluronic acid are in part dependent on its hydrophilic and hydrodynamic properties, which allow it to retain water and play a structural role. Indeed, hydrogels (cross-linked hydrophilic polymers) have been used as scaffolds to allow tissue repair or regeneration at sites of injury, being degraded by tissue enzymes after repair is completed.<sup>19</sup> Low molecular weight hyaluronic acid is pro-angiogenic, induces the formation of new blood vessels and activates a signal transduction pathway leading to endothelial cell proliferation and migration. In contrast, native high molecular weight hyaluronic acid is anti-angiogenic and will inhibit blood vessel formation.<sup>19</sup> Topical hyaluronic acid formulations are employed to treat recurrent aphthous ulceration of the oral mucosa<sup>21, 22</sup> with fast symptom relief, to which the dose-dependent anti-inflammatory activity of the compound<sup>23</sup> may also contribute.

Chondroitin sulphate is a natural glycosaminoglycan, present in the extracellular matrix surrounding cells, especially in the cartilage, skin, blood vessels, ligaments and tendons, where it forms an essential component of proteoglycans.<sup>24</sup> Current evidence shows that chondroitin sulphate fulfils important biological functions in

inflammation, cell proliferation, differentiation, migration, tissue morphogenesis, organogenesis, infection and wound repair.<sup>25</sup> These effects are related to the capacity of chondroitin sulphate to interact with a wide variety of molecules including (but not limited to) matrix molecules, growth factors, protease inhibitors, cytokines, chemokines and adhesion molecules *via* nonspecific/specific saccharide domains within the chains.<sup>25</sup> The compound is endowed with immune-modulatory,<sup>26</sup> anti-inflammatory<sup>25, 26</sup> and antioxidant<sup>27</sup> properties. Along with nonspecific interactions, chondroitin sulphate may display specific binding to bioactive molecules, such as pepsin. Peptic activity is indeed reduced both *in vitro*<sup>28</sup> and *in vivo*<sup>29, 30</sup>, and treatment of peptic ulcer with chondroitin sulphate has been attempted in the past.<sup>31</sup>

Poloxamer 407 (ethylene oxide and propylene oxide blocks) is a hydrophilic non-ionic surfactant, which shows thermo-reversible properties of the utmost interest in optimising drug formulation (fluid state at room temperature, facilitating administration and gel state above sol–gel transition temperature at body temperature, promoting prolonged release of pharmacological agents).<sup>32</sup> Poloxamer 407 formulations lead to enhanced solubilisation of poorly water-soluble drugs and prolonged release profile for many galenic applications.<sup>33</sup> The poloxamer 407 adhesive properties are used to lengthen residence time of agents in the gastrointestinal tract. Good adhesion in the oesophagus with efficient diffusion of the drug into the mucosa was observed in the mouse, by means of an optical fibre spectrofluorimetric method.<sup>32</sup>

According to European Council Directive 93/42/EEC,<sup>34</sup> the National Health Institute in Rome classified this bioadhesive formulation as class III medical device, intended for use in human beings for the purpose of treatment or alleviation of disease. Typically, the medical device function is achieved by physical means (including mechanical action, physical barrier, replacement of or support to organs or body functions).

An *ex vivo* experimental study on a swine model showed that perfusion of the oesophageal lumen with this medical device is able to prevent the increase in mucosal permeability induced by acid and/or pepsin.<sup>35</sup> With these data at hand, two double-blind, placebo-controlled studies demonstrated that short-term Esoxx treatment achieves a significant and quick symptom relief both in patients with erosive<sup>36</sup> or non-erosive reflux disease.<sup>37</sup>

In this prospective, double-blind, placebo-controlled trial the efficacy and safety of Esoxx, combined to acid suppression, vs. acid suppression alone, was evaluated in patients with NERD, diagnosed merely as endoscopy-

negative reflux disease. This was selected to mirror the clinical practice, outside the referral centres, where advanced investigations are not available.

## PATIENTS AND METHODS

Non-erosive reflux disease patients with typical reflux symptoms were enrolled in the study. They were of both sexes, and age ranged from 18 to 75 years. Two of the following symptoms, for example heartburn, acid regurgitation, retrosternal pain and acid taste in the mouth, should have been present from at least 3 months and at least three times per week in the month preceding the study screening visit. The diagnosis of NERD was based on the absence of macroscopic lesions of distal oesophageal mucosa at endoscopy,<sup>3, 4</sup> performed within 6 months from the screening visit, and by the positivity of a validated questionnaire (Reflux Disease Questionnaire, RDQ),<sup>38</sup> that is an RDQ score  $\geq 8$ .<sup>39</sup> In accordance with the NICE Guidelines<sup>40</sup> and to avoid interference with the rapid urease test,<sup>41</sup> routinely performed during endoscopy, patients were free from anti-secretory medication (either a PPI or an H<sub>2</sub>RA) for at least 2 weeks.

Exclusion criteria were the presence of erosive oesophagitis or Barrett's oesophagus, gastric or duodenal ulcer, previous gastric or major GI surgery, atopy or food intolerance, thyroid diseases, diabetes or metabolic syndrome. Moreover, pregnant, lactating or fertile women (without contraception) were also excluded.

## Study design

The study was multicenter, randomised, double-blind, placebo-controlled with parallel groups. Sixteen Italian hospitals were involved, and each of them obtained the approval of the respective ethical committee.

The trial was performed according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), guidelines for Good Clinical Practice (GCP)<sup>42</sup> and the Declaration of Helsinki (1996 version, amended October 2000).<sup>43</sup>

Patients eligible for the study gave informed and written consent and were asked to start a 15-day ( $\pm 2$  days) run in/wash out period, during which any (prescription or OTC) therapy was discontinued (visit 1). The only medications permitted were antacids or alginate-containing formulations in case of symptom occurrence. At visit 2, patients were randomised – according to a computer-generated sequence – to receive one standard dose of a PPI (30 min before breakfast) + 10 mL (1 stick) of Esoxx One (single dose stick formulation) or placebo (with the same

taste and viscosity, packed in identical, sequentially numbered, containers) q.d.s., that is 1 h after each daily meal, and at bedtime for a 14-day treatment period. The allocation sequence was generated by LB Research (contract research organisation), while enrolment and assignment of participants to a given treatment were performed by the principal investigator of the study centres (see Appendix 1). During the study period, daily symptom diaries, recording the presence or absence of each symptom during the day and the night, were filled by each patient. Before (visit 2) and after this short course of therapy (visit 3), frequency and severity of NERD symptoms were evaluated using the same RDQ questionnaire. Health-related quality of life (HRQL) was also assessed using the SF-36 questionnaire.<sup>44</sup> Both questionnaires were administered by physicians, who were unaware of the treatment given. Results of each item were compared with those of published data for the Italian normative sample.<sup>45</sup> Pre- and post-treatment results for each item were also compared. The study design and the detailed assessment schedule can be found in the Table S1.

Safety and tolerability were assessed by recording all the adverse events, defined as any unfavourable or unintended symptom and/or sign, considered to be casually related to the drug(s) used in the study. The palatability was evaluated after each drug administration, according to a 4-item scale (excellent, good, irrelevant and bad). Hence, there were 4 per day  $\times$  15 days evaluations for each patient.

Finally, patients' compliance was defined as the percentage of the test drug used, obtained by counting the returned medications at visit 3. A treatment compliance of 80–120% was considered acceptable.

The European Clinical Trials Database (EudraCT), launched by the European Medicines Agency (EMA), does not accept clinical trials investigating medical devices, but refers to the procedures in place in the Country, where the clinical trial is conducted. Accordingly, the Clinical Trial Protocol was registered (Protocol code: Esoxx-NERD/001/2012) at the Italian Ministry of Health, and the beginning of the trial (i.e. the inclusion of the first patient), as well as the end of the trial (i.e. the last evaluation of the last included patient), was notified to the regulatory authorities.

### Statistical analysis

The primary endpoint was the treatment efficacy analysis, which was calculated as the proportion of patients with at least 3-point reduction of the total symptom score (TSS). This was calculated by collecting and computing the intensity of each patient's symptom (on the

basis of the RDQ questionnaire at the final visit) and comparing it with the baseline values, obtained at the end of the run in/wash out period (visit 2). Typical symptoms were evaluated according to a 5-degree Likert scale<sup>46</sup>: 0 = no symptom, 1 = poorly troublesome symptoms, 2 = troublesome symptoms, 3 = very troublesome symptoms, interfering with daily activities, 4 = intolerable symptoms, not permitting any daily activity.

There were four different secondary endpoints: (i) number of patients with 50% reduction of TSS at final visit, (ii) number of patients with TSS reduction at the final visit, (iii) change TSS after treatment and (iv) HRQL physical and mental items according to the SF-36 questionnaire, which were calculated via a web-based program<sup>47</sup> and presented as radar plots or spidergrams.<sup>48</sup> Changes in the severity and frequency of each symptom (heartburn, acid regurgitation, retrosternal pain, acid taste in the mouth) were also evaluated.

The intention-to-treat (ITT) population included all randomised patients, who took at least one dose of medication while per protocol (PP) analysis was performed on all randomised patients, who concluded the treatment, with an adequate compliance rate and without any protocol violation. The former analysis was used to evaluate the primary endpoint and the latter for both primary and secondary endpoints. The safety population included all randomised patients, who took at least one dose of the study drugs.

Chi-squared and Fisher's exact test, two tails, were used to compare percentages of values for primary and secondary endpoints, while arithmetic means and frequencies were assessed by means of 95% confidence intervals (CIs).<sup>49</sup> All the calculations were performed using the PRISM 6.0 software (GraphPad, San Diego, CA, USA), running on a MAC.

The sample size was calculated on the basis of the reduction of NERD TSS by 3 points at final visit and assuming a rate of 10% improvement in the placebo group and 30% in the Esoxx arm. A power level of 80% with a significance value  $\leq 0.05$  (two-sided Fisher's exact test) required a sample size of 70 patients for each group. Taking into account a 12% of non-evaluable patients, the sample was raised to 80 patients. The estimation was made, using the STATA (Version 13, StataCorp LP, College Station, TX, USA) for MAC.

### RESULTS

In the 16 centres involved in the study, 172 NERD patients were screened and 154 out of them were randomised to treatment, 76 in the Esoxx group and 78 in

the placebo one. Among them, 18 patients were considered dropouts for various reasons: eight for adverse events, two for treatment failure and eight denied consent (Figure 1).

Table 1 shows the baseline demographics and the clinical characteristics of the groups studied, in the ITT population. There were no statistical differences among the different characteristics of the recruited patients in the two arms.

The compliance, defined as mean number ( $\pm$ s.d.) of sticks taken, was similar ( $P = \text{NS}$ ) in the two arms of treatment, that is  $90.9 \pm 22.9$  vs.  $90.2 \pm 20.7$  in the Esoxx and placebo groups, respectively.

As regards the primary endpoint, Table 2 (ITT analysis) shows that the proportion of patients with TSS

reduction of at least 3 points at final visit was higher in the Esoxx than in the placebo group and the difference was always significant. Also the proportion of patients with 50% TSS reduction at visit 3, as secondary endpoint, resulted to be significantly higher ( $P < 0.042$ ) in the Esoxx (38.2%) than in the placebo (23.1%) group (Table 2). In addition, number of patients with TSS reduction at the final visit was significantly higher in Esoxx than in placebo arm ( $P < 0.026$ ). Finally, TSS after treatment improved more with Esoxx than with placebo treatment ( $P < 0.011$ ). Similar results were obtained in the PP population (Table S2). As shown in Table 3, all the symptoms evaluated subsided with both treatments, but the amelioration of heartburn and especially regurgitation was more marked with Esoxx combined with

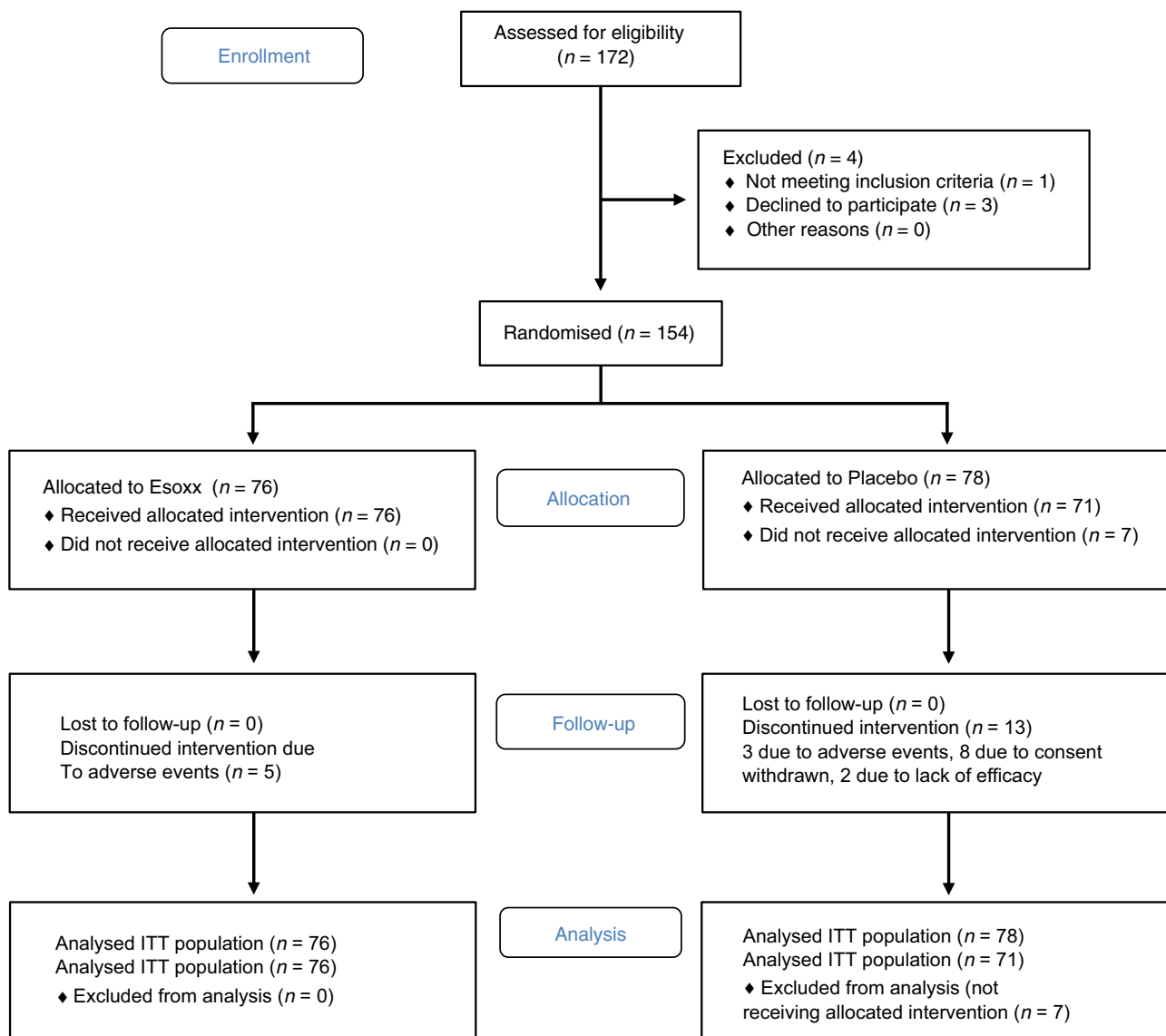


Figure 1 | Consort 2010 flow diagram.



**Table 1** | Baseline characteristics of NERD patients receiving Esoxx or placebo, combined with PPIs

	Esoxx (n = 76)	Placebo (n = 78)	P value
Female, N (%)	48 (63.2%)	46 (59.0%)	NS
Age (years), mean $\pm$ s.d.	45.45 $\pm$ 14.98	45.51 $\pm$ 13.37	NS
Range (min–max)	18–81	24–75	
BMI (kg/m <sup>2</sup> )	23.87 $\pm$ 3.10	23.77 $\pm$ 3.23	NS
GERD total symptom score	7.30 $\pm$ 2.4	7.19 $\pm$ 2.6	NS
Proportion of patients with $\geq 3$ GERD symptoms (%)	44.0	41.0	NS
Heartburn	84.2	85.9	
Retrosternal pain	53.9	49.3	
Acid regurgitation	69.7	66.2	
Acid taste in the mouth	60.5	59.2	
Past treatment with PPIs (%)	56.6	64.8	NS
Past treatment with other anti-GERD therapies (%)	23.7	29.6	NS

**Table 2** | Effect of Esoxx, combined with PPI therapy, on primary and secondary endpoints in patients with NERD: ITT analysis

Trial endpoints	PPI + Esoxx		PPI + Placebo		P value
	n/N	%	n/N	%	
Primary					
No of patients with TSS reduction of at least 3 points	40/76	52.6	25/78	32.1	0.01
Secondary					
No of patients with 50% reduction of TSS	29/76	38.2	18/78	23.1	0.042
No of patients with TSS reduction at final visit	60/76	78.9	44/78	56.4	0.003
TSS ( $\pm$ s.d.) before and after treatment	Before	After	Before	After	
	8.53 $\pm$ 2.6	5.42 $\pm$ 2.1	8.03 $\pm$ 2.7	6.49 $\pm$ 2.6	
Change ( $\pm$ s.d.) in TSS	–3.11 $\pm$ 3.1		–1.54 $\pm$ 3.0		0.002

TSS, total symptom (heartburn, retrosternal pain, regurgitation, acid taste) score.

PPIs. The therapeutic gain with Esoxx was 20.5%, 15.3% and 10.2% for TSS (symptom severity), heartburn and regurgitation incidence, respectively.

Finally, the quality of life, evaluated by means of the SF-36 items, improved with both treatments (Figure 2). Indeed, 2 weeks after therapy the SF-36 items become closer to those of the Italian normative sample.<sup>45</sup> However, the improvements in General Health Perception and the Social Function items were significantly ( $P < 0.01$  and  $P < 0.02$ , respectively) better after Esoxx plus PPI therapy.

The safety of Esoxx was very good, as the total number of adverse events was similar to that of placebo and there were no serious adverse events in any treatment arms (Table 4). The most frequent manifestations pertained to the gastrointestinal tract (nausea, flatulence, bloating, dyspepsia, etc.) and respiratory organs (cough, rhinitis, pharyngeal disorders) (Table 5).

On the basis of the total number of evaluations collected ( $n = 7230$ ), in 92% of Esoxx administrations, palatability was considered acceptable, independently of

the intake time (be it during the day or at bedtime) while the same held true for 90% of placebo administrations ( $P = \text{NS}$ ). The distribution of these evaluations is shown in Figure 3.

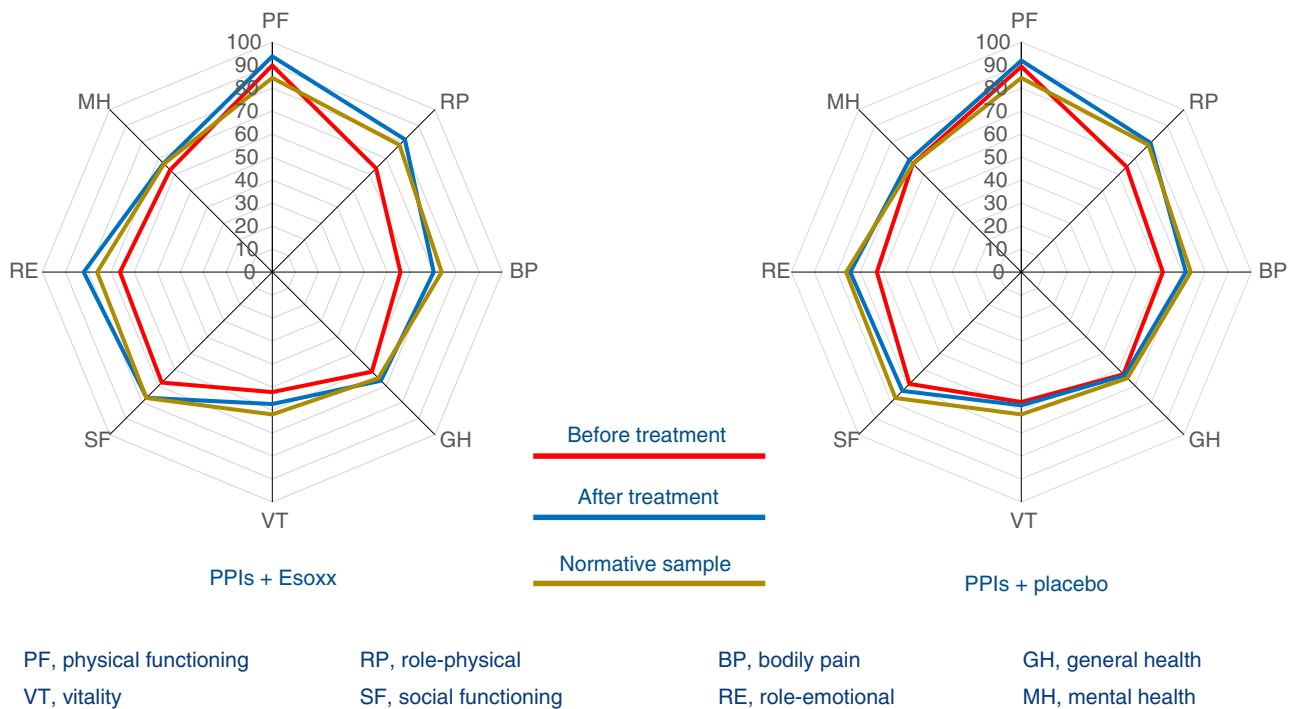
## DISCUSSION

The results of this study show that, when mucosal protection is added to acid suppression, a significantly higher number of NERD patients obtained symptom relief with combination therapy. Indeed, both the primary and secondary endpoints were achieved in a larger proportion of subjects.

Although PPIs are effective in obtaining symptom relief in both erosive and NERD,<sup>50</sup> their efficacy for the relief of regurgitation is modest, and considerably lower than that achieved for heartburn.<sup>51</sup> In addition, although not as frequent as previously suggested,<sup>7</sup> PPI-refractory heartburn, occurring more commonly in NERD than in erosive disease, does exist. Some 20% (range 15–27%) of correctly diagnosed and

**Table 3 | Effect of Esoxx, combined with PPI therapy, on (a) severity and (b) frequency of GERD symptoms in patients with NERD: ITT analysis**

	PPI + Esoxx, mean score ± s.d.			PPI + placebo, mean score ± s.d.			P value Esoxx vs. placebo
Symptom	Before therapy	After therapy	Adjusted mean change (95% CI)	Before therapy	After therapy	Adjusted mean change (95% CI)	
(a)							
Heartburn	1.80 ± 1.1	0.72 ± 0.8	−1.131 (−1.340 to −0.922)	1.99 ± 1.0	1.09 ± 1.0	−0.836 (−1.034 to −0.638)	0.0319
Regurgitation	1.84 ± 1.1	0.64 ± 0.8	−1.095 (−1.280 to −0.911)	1.53 ± 1.1	0.94 ± 1.0	−0.685 (−0.861 to −0.509)	0.0009
Retrosternal pain	1.36 ± 1.2	0.42 ± 0.7	−0.852 (−1.023 to −0.682)	1.15 ± 1.2	0.59 ± 0.8	−0.612 (−0.775 to −0.449)	0.0323
Acid taste in the mouth	1.53 ± 1.1	0.63 ± 0.8	−0.754 (−0.968 to 0.541)	1.3 ± 1.1	0.8 ± 1.0	−0.494 (−0.696 to −0.291)	0.0623
(b)							
Heartburn	3.08 ± 1.7	1.38 ± 1.5	−1.719 (−2.083 to −1.354)	3.23 ± 1.5	1.94 ± 1.6	−1.229 (−1.578 to −0.883)	0.0408
Regurgitation	2.92 ± 1.7	1.23 ± 1.5	−1.562 (−1.892 to −1.233)	2.60 ± 1.8	1.63 ± 1.7	−1.021 (−1.332 to −0.710)	0.0128
Retrosternal pain	2.14 ± 1.8	0.82 ± 1.3	−1.232 (−1.511 to −0.952)	1.86 ± 1.7	1.03 ± 1.3	−0.896 (−1.163 to −0.630)	0.0676
Acid taste in the mouth	2.57 ± 1.7	1.16 ± 1.5	−1.285 (−1.640 to 0.930)	2.38 ± 1.8	1.53 ± 1.7	−0.876 (−1.213 to −0.540)	0.0790

**Figure 2 | HRQL measured in NERD patients before and after 2-week treatment with Esoxx or Placebo combined to PPIs. Note that, after treatment, the SF-36 items are close to those of the Italian normative sample.**

appropriately treated patients do not respond to PPI therapy at standard doses.<sup>52</sup>

Various underlying mechanisms have been shown to contribute to the failure of PPI treatment. They include patient-related (e.g. lack of compliance), physician-related (e.g. misdiagnosis) and drug-related (e.g. short duration of action) mechanisms.<sup>53, 54</sup> At the present time, much

current research is focused on weakly acidic reflux<sup>55</sup> and oesophageal hypersensitivity.<sup>56</sup> The pH-impedance technique has been increasingly used to explore the underlying pathophysiology in PPI-resistant patients. Several groups of investigators have indeed shown that weakly acidic reflux plays a major role in PPI-resistant erosive and non-erosive disease.<sup>57</sup> pH-impedance monitoring has also

**Table 4 |** Adverse events in NERD patients, included in the ITT analysis, receiving PPI + Esoxx or PPI + placebo

	Esoxx (n = 76)	Placebo (n = 71)	P value
Total number of unique AEs	32	14	NS
Total number of AEs	35	20	NS
Total number of patients with at least one AE	18 (23.7)	11 (15.5)	NS
Total number of unique drug-related AEs	23	13	NS
Total number of drug-related AEs	24	19	NS
Total number of patients with at least one related AEs	13 (17.1)	10 (14.1)	NS
Total number of serious AEs	0	0	NS
Total number of patients with at least one AE leading to discontinuation	5 (6.6)	3 (3.8)	NS

Values within parenthesis are expressed as percentage. AE, adverse event.

**Table 5 |** Patients, treated with PPI combined with Esoxx or placebo, with at least one TEAEs classified for system organ class (SOC) – safety analysis

SOC	Esoxx (n = 76)	Placebo (n = 71)
Patients with at least one TEAE	18 (23.7)	11 (15.5)
Gastrointestinal disorders	13 (17.1)	7 (9.9)
Respiratory, thoracic, mediastinal disorders (cough, rhinitis, throat irritation, pharyngeal disorders)	4 (5.3)	1 (1.4)
Nervous system disorders (dysgeusia, headache, migraine)	3 (3.9)	–
Cardiac disorders (palpitations, tachycardia)	1 (1.3)	1 (1.4)
Ear and labyrinth (vertigo)	1 (1.3)	–
General disorders (hypertension)	1 (1.3)	–
Infections and infestations	1 (1.3)	3 (4.2)

Values within parenthesis are expressed as percentage.

allowed identification of a previously unknown subgroup of patients, namely those with normal oesophageal pH-impedance recording, but a positive association between symptoms and non-acidic reflux episodes, that is patients

who are hypersensitive to non-acidic reflux.<sup>57</sup> Finally, this methodology has enabled a better differentiation between patients with NERD and those with functional heartburn.<sup>58</sup> It is therefore evident that different patient subgroups belong to NERD, which is indeed an umbrella term. Among them, only patients with true NERD or acid hypersensitive oesophagus (now called reflux hypersensitivity, according to the Rome IV criteria<sup>59</sup>) are expected to display a satisfactory symptomatic response to acid suppression therapy with a PPI. On the contrary, subjects hypersensitive to non-acid reflux or those with functional heartburn (which – together with reflux hypersensitivity – does not pertain anymore to the realm of GERD) will obviously be nonresponsive to anti-secretory drugs.<sup>60</sup>

In patients with NERD, who are refractory to a correctly performed PPI therapy, the lack of symptom relief could be due to persistence of microscopic mucosal alterations induced by weakly acidic reflux,<sup>61</sup> by pepsin or other components of the refluxate<sup>62</sup> and underlined by an impaired mucosal integrity.<sup>63</sup> Current pharmacologic approaches to address this clinically challenging condition are limited. Reflux inhibitors represent a promise unfulfilled,<sup>64</sup> effective prokinetics are lacking<sup>65</sup> and antidepressants, despite being effective in selected patients,<sup>66</sup> give rise to adverse events in up to 32% of patients.<sup>67</sup>

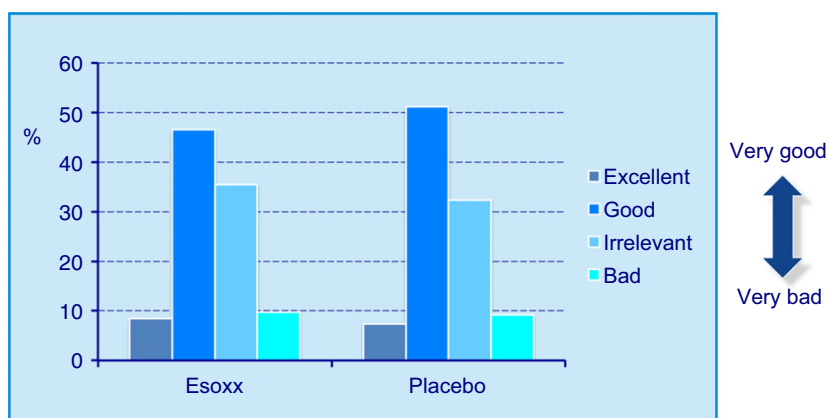
A better approach to patients with NERD should be therefore making a more precise diagnosis, by adding a functional evaluation (e.g. pH or pH-impedance recording) to negative endoscopy. When this has been done, the estimated complete symptom response rate after PPI therapy appeared comparable to that observed in patients with GERD.<sup>68</sup> Including biopsy (and subsequent histology) of the ‘macroscopically normal’ mucosa during endoscopic examination<sup>61</sup> would be ideal. It is evident, however, that this approach, being time-consuming and costly, is not achievable in the everyday clinical practice.

An alternative, easier, approach could be combination therapy, that is adding drugs with different mechanism (s) of action to PPIs. Up to now, only irsogladine (a mucosal protective compound)<sup>69</sup> and alginate-containing formulations<sup>70, 71</sup> – given as add-on medications – proved to be capable of improving symptom control in NERD patients. The addition of mosapride (a prokinetic compound) to PPIs does not add any benefit<sup>72, 73</sup> unless NERD patients display a delay in gastric emptying.<sup>74</sup>

The mucosal protective device, Esoxx, was shown to be capable of achieving a significant and quick symptom relief in patients with NERD in this and a previous trial.<sup>37</sup> Its amelioration of regurgitation severity and frequency is of clinical interest, taking into account the negligible effect



**Figure 3 |** Palatability assessment of Esoxx or placebo formulations, used in the present study. Distribution of the 60 evaluations for each patient.



PPIs have on this cardinal symptom of reflux disease.<sup>51, 63</sup> As shown by a small study,<sup>36</sup> this formulation may well be effective also in patients with erosive disease, in whom its protective and reparative properties would favour healing of oesophageal mucosal lesions.

The synergistic effect of Esoxx with PPIs, shown in this study, suggests that mucosal protection, routinely added to acid suppression, could extend to a larger number of patients with NERD both symptom relief and improvement of HRQL, thus reducing the incidence of treatment failures. PPIs achieve a symptom relief, which increases over time both in erosive and non-erosive disease. This has been further shown by the studies comparing PPIs (namely esomeprazole) with P-CABs (namely linaprazan).<sup>75, 76</sup> It may well be that this combined approach achieves at 2 weeks the same symptom relief, obtained with PPIs at 4 weeks. However, for those patients asking for quick symptom relief, this time-dependent therapeutic gain could be worthwhile from their own perspective.

The present study has intrinsic limitations. As functional investigation (i.e. pH-impedance recording) was not performed, the population studied included patients with functional heartburn and reflux hypersensitivity. In addition, although adequately powered to show a significant effect, this was a relatively small trial. A larger study in patients with PPI-resistant NERD as well as a trial in patients with extra-oesophageal symptoms is worthwhile.

Despite recent research has established the sites and mechanisms underlying oesophageal mucosal defence, its enhancement is very rarely pursued in clinical practice. Drugs able to strengthen mucosal defence do exist, but they have not been studied in well designed clinical trials.<sup>63</sup> Due their high efficacy in reflux disease, it is unlikely that these drugs represent a real alternative to PPIs. However, their use in less severe disease or as add-on

medications to PPIs could be useful. Furthermore, used in the long term, these mucosal protective compounds might prolong remission and delay relapse.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Study design and assessment schedule.

**Table S2.** Effect of Esoxx, combined with PPI therapy, on primary and secondary endpoints in patients with NERD: PP analysis.

## AUTHORSHIP

*Guarantor of the article:* C. Scarpignato.

*Author contributions:* According to the International Committee of Medical Journal Editors (ICMJE), Carmelo Scarpignato and Vincenzo had full access to all of the data in the study and take the responsibility for the integrity of the data. Carmelo Scarpignato, Vincenzo Savarino performed study concept and study design and drafted the manuscript; Carmelo Scarpignato, Vincenzo Savarino and Fabio Pace analysed and interpreted the data and critically revised the manuscript for important intellectual content; LB Research (Claudio Iannacone) and Carmelo Scarpignato performed statistical analysis; Carmelo Scarpignato obtained funding; LB Research (Sara Bellasio) performed administrative, technical or material support; and Antonella Ferrieri performed study supervision. All authors approved the final version of the manuscript.

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*Declaration of personal interests:* Vincenzo Savarino is member of the Speakers' Bureau of Alfa Wassermann, the manufacturer of Esoxx. Fabio Pace has no conflict of interests to disclose. Carmelo Scarpignato is member of the Speakers' Bureau and of the Advisory Board of Alfa Wassermann.

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