Reflux disease and achalasia: Failure of the gatekeeper
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Chapter 2

Study on the mechanisms underlying PPI resistance in GERD patients

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Submitted
ABSTRACT

Background and aims: Approximately 30% of patients with gastroesophageal reflux disease (GERD) have symptoms resistant to treatment with proton pump inhibitors (PPIs). Several mechanisms such as esophageal hypersensitivity, increased mucosal permeability and possibly the position of the gastric acid pocket have been suggested to underlie a partial response to PPI. To what extent these mechanisms interact and contribute to PPI resistant symptoms has however not been investigated.

Methods: In eighteen GERD patients (9 PPI responders and 9 PPI partial responders), esophageal sensitivity, mucosal permeability and postprandial reflux parameters were determined during PPI use. Esophageal sensitivity for distension was measured by gradual balloon inflation at 5 and 15 cm above the LES. Mucosal permeability of 4 esophageal biopsies was determined in Ussing chambers by measuring the transepithelial electrical resistance (TEER) and transmucosal flux of fluorescein. Postprandial reflux parameters were determined using concurrent high-resolution manometry/pH-impedance following a standardized meal. In addition, the acid pocket was visualized using scintigraphy.

Results: PPI partial responders had more reflux episodes with a higher mean proximal extent, compared to PPI responders. Additionally, PPI partial responders were more sensitive to balloon distension, both in the upper and the lower esophagus. No difference in the rate of postprandial acid reflux or in the pH of the acid pocket (PPI responders 3.7 ± 0.7 vs. PPI partial responders 4.2 ± 0.4 p=0.54) was observed. Additionally, the position of the acid pocket was similar. Permeability of esophageal mucosa did not differ, as demonstrated by a similar TEER and flux of fluorescein.

Discussion: Mucosal permeability and the position of the acid pocket are similar in PPI partial responders and responders and are therefore less likely to explain persistent symptoms. In contrast, esophageal sensitivity to distension and the number of reflux episodes with a higher proximal extent are increased in PPI partial responders compared to PPI responders. Based on these results, we suggest that PPI resistant symptoms are most likely explained by increased proximal reflux in a hypersensitive esophagus.
**Introduction**

Gastroesophageal reflux disease (GERD) is a common chronic condition, with some 20 per cent of the population in Western countries experiencing typical symptoms such as heartburn or regurgitation at least once a week. The current choice of treatment is undoubtedly acid suppression, especially as healing of the mucosa is achieved within 8 weeks of treatment with proton pump inhibitors (PPIs) in the large majority of patients (>90%). However, in up to 30% of patients, PPI therapy fails to completely resolve symptoms. Additionally, it appears that only less than 50% of patients with GERD are satisfied with their medical treatment.

Given the high prevalence of GERD, PPI resistant symptoms represent a significant clinical problem and press a high burden on current health care.

The cause of symptoms unresponsive to PPIs is incompletely understood. To this end, Zerbib et al studied 1273 reflux episodes during PPI use and their relationship to symptoms. Of note, up to 60% of PPI resistant symptoms were not associated with acid reflux episodes, a phenomenon detected in 50% of patients. In the remaining patients, symptoms were mainly associated to weakly acidic reflux episodes with proximal extent of the refluxate as most important determinant of symptom perception.

Several mechanisms that may contribute to symptom perception and thereby to PPI resistant symptoms have been suggested. First, increased perception of a variety of stimuli, including acid, esophageal distention, electrical stimulation and temperature, also referred to as visceral hypersensitivity, has been repeatedly reported. Second, impaired mucosal integrity or dilated intercellular spaces (DIS) have been proposed to underlie PPI resistant symptoms. The apical membranes and junctional complexes of the cell prevent the diffusion of noxious refluxed luminal contents from penetrating into the esophageal mucosa. Acid, bile acids and acid-pepsin can however damage mucosal integrity, potentially contributing to increased perception and symptoms such as heartburn. Calabrese et al demonstrated that DIS, a marker of impaired mucosal integrity, can be restored by PPI treatment, a finding associated with relief of symptoms.

Finally, we recently reported that the gastric acid pocket is a major player in the pathogenesis of GERD. The contribution of differences in acidity, position or size of the acid pocket in PPI resistant symptoms however remains unclear.

Based on the above, visceral hypersensitivity, impaired mucosal integrity and differences in the acid pocket may all contribute to PPI resistant symptoms in GERD patients. To what extent these mechanisms are present within the same patient or are rather predominant and characteristic for a certain subpopulation of PPI partial responders has not been addressed in detail. Therefore, we determined esophageal sensitivity and mucosal integrity, visualized the acid pocket and assessed postprandial reflux parameters in PPI responders and partial responders to obtain a better and
integrated picture of the mechanisms underlying PPI resistant symptoms in GERD patients. Clearly, this insight is of great importance to better target these symptoms and develop more effective treatments.

METHODS

Patients
We included 18 patients with GERD, of whom 9 were PPI responders (5 males, 59 years) and 9 were patients with refractory symptoms on PPI (2 males, 52 years). The latter category of patients was classified as partial PPI responders. A diagnosis of GERD was based on the observation of esophagitis during endoscopy and/or a pathological acid exposure, both in combination with typical reflux symptoms off PPI. In GERD patients with complete response, typical reflux symptoms were absent during PPI use. For partial PPI responders the relation between reflux symptoms and reflux episodes had to be proven by a positive symptom association probability (>95%). The study was approved by the Medical Ethics Committee of the Academic Medical Center. Written informed consent was obtained from all subjects before enrolment in the study.

Study protocol
Study subjects underwent 3 measurements on 3 different study days with at least 3 days in between. (Figure 1.) On the first study day esophageal sensitivity for balloon distension and infusion of weakly acid (pH 4.0) and saline (pH 7.0) was determined. Sensitivity to distension and infusions were tested in the distal and proximal esophagus. For all distensions and infusions patients reported pain/discomfort using visual analogue scale (VAS) scores, on a scale from 0 to 100 mm.

On the second study day we performed concurrent high resolution manometry (HRM) and pH-impedance monitoring to detect postprandial reflux episodes. In addition, 350 MBq technetium-99m (\(^{99m}\)Tc)-pertechnetate was injected intravenously to scintigraphically visualize the acid pocket\(^ {13,14} \). Then, a HRM catheter and pH-impedance catheter were inserted transnasally. After positioning of the catheters, patients were positioned in upright position in front of the scintigraphy camera. First, a baseline fasting recording was obtained during 5 minutes. Then patients consumed a standardized meal in 10 minutes consisting of 200 ml orange juice and two pancakes with jam (510 kcal). After the meal, scintigraphic, HRM and pH-impedance recordings were performed for 105 minutes.
Figure 1 | The 3 study days are presented in this image. Figure 1.1 demonstrates the esophageal sensitivity measurement. A manometry catheter with a balloon and infusion port is introduced. Sensitivity for balloon distension and infusion of neutral (pH 7.0) and weakly acid (pH 4.0) solution is tested at 5 cm and 15 cm above the LES. Figure 1.2 represents a schematic view of the second study day set-up. Two radionuclide markers are attached to the HRM catheter (blue dots), which can be observed on the scintigraphic image, on the proximal and distal end of the acid pocket. A representative image of the HRM and pH-impedance recording is shown underneath, with a mixed reflux episode during a TLESR in a patient with a hiatal hernia. The LES is marked with A, and the upper and lower borders of the crural diaphragm are marked with B and C respectively. On the third study day (Figure 3.3) 4 esophageal biopsies are mounted in Ussing chambers. Paracellular permeability is determined by measuring fluorescein concentration on the serosal side 30 minutes after adding 0.5 mg/mL fluorescein on the mucosal side. Transepithelial electrical resistance is determined by using Ohm’s law after measuring voltage deflection induced by a bipolar constant current of 20 µA.

On the third study day patients underwent upper endoscopy with a therapeutic endoscope (Olympus Endoscopy, Tokyo, Japan). After regular inspection of the duodenum, stomach and esophagus, 4 large jaw biopsies (3.7 mm) were taken 5 cm above the Z-line. Mucosal integrity of these biopsies was determined in Ussing chamber experiments. All measurements were performed during PPI use.

**Esophageal sensitivity**

Esophageal sensitivity was determined using a manometry catheter with an inflatable balloon and a side hole for infusion (Figure 1). The proximal border of the lower esophageal sphincter was determined based on the manometric recordings of the esophagus, LES and stomach obtained.
using a perfused 10-channel silicone rubber assembly (DentSleeve International Ltd, Mississauga, Ontario, Canada). Sensitivity was determined in the distal and proximal esophagus, defined as 5 and 15 cm above the proximal border of the LES. First, the balloon was inflated to 0, 5, 10 and 15 mL of air in a random order. Subsequently, 150 mL of neutral and weakly acid (pH 4.0) solution were infused for 3 minutes (50 mL per minute). Patients reported a VAS score to pain or discomfort ranging from 0 to 100 for all balloon sizes and infusions in the proximal and distal esophagus. For balloon distension, the areas under the curve and the mean VAS score of the 4 distensions were determined in each patient, and compared for the 2 groups. The VAS scores of weakly acid infusion in the proximal and distal esophagus were compared for the 2 groups.

**Postprandial reflux and acid pocket protocol**

We performed concurrent high resolution manometry (HRM) and pH-impedance monitoring to detect postprandial reflux episodes, and scintigraphy to determine the size and position of the postprandial acid pocket.

High resolution manometry was performed using a 21 lumen water perfused HRM catheter (MMS, Enschede, the Netherlands). Eleven distal side holes were positioned at 1 cm intervals, and the 10 proximal side holes were spaced at 3 cm intervals. The side holes were perfused with distilled water at 0.15 mL/min, using a pneumohydraulic capillary perfusion pump (MMS, Enschede, the Netherlands) and hydraulic flow restrictors. The working channel had a diameter of 0.9 mm with the opening located at the distal tip of the catheter. Pressure sensors were zeroed before insertion and data was collected and analyzed with a MMS Solar system (MMS, Enschede, the Netherlands). We used a standard combined pH-impedance catheter (Unisensor, Attikon, Switzerland), containing 6 pairs of impedance electrodes and 1 ISFET pH sensor that allowed impedance recordings at 3, 5, 7, 9, 15 and 17 cm above the upper border of the LES and pH recording at 5 cm above the LES. The HRM-catheter was positioned with the 3 most distal sensors located in the stomach, and the pH-sensor of the pH-impedance catheter was positioned 5 centimetres above the upper border of the LES.

Thirty minutes prior to the start of these measurements, 350 MBq of $^{99m}$Tc-pertechnetate was injected intravenously. After a meal, $^{99m}$Tc-pertechnetate is secreted by parietal cells similar to chloride ions. We previously validated that the postprandial pooling of $^{99m}$Tc-pertechnetate in the proximal stomach represents the gastric acid pocket. The acid pocket can therefore be observed on scintigraphy, also during PPI use. After the standardized meal recordings were performed for 105 minutes. Patients were studied in the upright position and scintigraphic images were made in posterior view. At the end of the measurements the acid pocket was aspirated through the working channel of the HRM-catheter, guided by the radionuclide marker on the distal tip of the catheter on scintigraphy.
Dynamic scintigraphic images were acquired on a gamma camera system (Diacam; Siemens Medical Solutions, IL, USA), equipped with a low-energy all purpose collimator. Dynamic recordings were made for 2 hours (720 views, 10 s/view, 120 min total acquisition time). The acid pocket was observed as a pooling of nuclear activity in the proximal stomach. Scintigraphic images were processed on a Hermes processing station (Hermes Medical Solutions, Stockholm, Sweden) for further analysis.

To determine the exact location of the pocket relative to the crural diaphragm, 2 sealed markers impregnated with topical $^{99m}$Tc-pertechnetate were attached to the HRM-catheter. The nuclear markers attached to the HRM catheter were observed as clear dots on the proximal and distal side of the acid pocket. We detected each reflux episode and determined the acidity of the refluxate and the proximal extent. Subsequently we used the two markers as demonstrated in Figure 1.2 to localize the acid pocket relative to the crural diaphragm, similar to earlier studies. The position of the acid pocket was classified as under, at the level or above the diaphragm.

**Ussing chamber experiments**

During endoscopy 4 biopsies were obtained using a large biopsy forceps for Ussing chamber studies. The biopsies were taken at 5 cm from the Z-line. In patients with erosive reflux disease the biopsies were taken at macroscopically normal mucosa. The biopsies were immediately transferred to Ussing chambers in ice-cold oxygenated modified Meyler buffer composed of 105 mM NaCl, 4.7 mM KCl, 1.3 mM CaCl$_2$, 1.0 mM MgCl$_2$, 20.0 mM NaHCO$_3$, 0.4 mM Na$_2$HPO$_4$, 0.3 mM Na$_2$HPO$_4$ and 10.0 mM HEPES, pH 7.4. Biopsies were mounted in special biopsy holders with a diameter of 2 mm and a square area of 0.0314 cm$^2$. Tissue was bathed in the modified Meyler buffer, while being continuously gassed with carbogen (95% O$_2$ - 5% CO$_2$). The tissue was kept at 37 °C using hot water jackets.

Two sets of electrodes connected to a dual voltage clamp (World Precision Instruments, Berlin, Germany) were used to short-circuit the tissue, allowing the direct measurement of voltage deflection induced by a bipolar constant current of 20 µA. The transepithelial electrical resistance (TEER) was calculated according to Ohm’s law. Transepithelial permeability for small molecules was used as a second measure of mucosal integrity, by adding fluorescein (376 Da) at a concentration of 0.5 mg/ml to the mucosal side of the biopsy and measuring it at the serosal side. The serosal bath was sampled before the addition of fluorescein, to serve as a zero value.

After a 15 minute acclimatisation period, we performed two mucosal integrity tests in a random order. For the first test we replaced the luminal buffer with a solution of modified Meyler buffer containing fluorescein at a concentration of 0.5 mg/ml. After thirty minutes TEER and the serosal side was sampled to determine the flux of fluorescein. In the other integrity test, we aimed to
study the effect of short acid exposure on esophageal mucosa. For this purpose we replaced the luminal buffer with acidified modified Meyler buffer (pH 2.0) for five minutes. We subsequently replaced luminal and serosal buffer for modified Meyler buffer containing fluorescein (0.5 mg/mL) and Meyler buffer respectively. After 30 minutes the serosal bath was sampled once more.

The fluorescein concentration in the samples was measured with a fluorescence plate reader (BioTek Synergy, BioTek, Winooski, VT, USA) using an excitation wavelength of 485 nm and an emission wavelength of 538 nm. The transepithelial permeability of tissue is expressed as nmol/cm². Transepithelial permeability and TEER were compared for the two groups of patients, and for with and without acid exposure.

**Statistical analysis**
Statistical analysis was performed using SPSS 18.0 (IBM Corporation, Somers, NY, United States). Data are presented as median [IQR]. Data were tested using a Mann-Whitney U test in case of unpaired data, and the Wilcoxon signed rank test in case of paired data. Comparison of proportions was performed using Fisher’s exact testing. Pearson’s and Spearman’s correlation were used for correlations, in case of parametric and non-parametric data respectively. All p-values were two-tailed and a p-value < 0.05 was considered as statistically significant.

**RESULTS**

**Patients**
In all 18 patients esophageal sensitivity measurements and the postprandial reflux protocol were completed. In 2 patients (1 responder, 1 partial responder) endoscopy could not be performed, such that no biopsies were available for Ussing experiments.

No significant difference was found in ages and sex between complete and partial responders (Age: 59 ± 4.9 year vs. 52 ± 6.4 years, p=0.39; Sex 5 male vs. 2 male, p=0.34). PPIs used were omeprazole, pantoprazole and esomeprazole (8, 5 and 5 patients respectively), and dosage varied from 20 mg to 40 mg and to 40 mg bid (1, 7 and 8 patients respectively).

**Mucosal integrity**
No differences were observed in basal TEER, TEER after 30 minutes and mucosal permeability of fluorescein between PPI responders and partial responders (Table 1). We did observe a good inverse correlation between basal TEER and the flux of fluorescein after 30 minutes (r=-0.63, p=0.01) demonstrating that a lower epithelial resistance leads to a more permeable mucosa.
Partial PPI failure in GERD | Chapter 2

Partial PPI responders (n=8) | PPI responders (n=8) | P

<table>
<thead>
<tr>
<th>Basal TEER (Ω.cm(^2))</th>
<th>87 [63-103]</th>
<th>84 [63-97]</th>
<th>0.86</th>
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</thead>
<tbody>
<tr>
<td>30 minutes TEER (Ω.cm(^2))</td>
<td>87 [60-107]</td>
<td>81 [64-99]</td>
<td>0.92</td>
</tr>
<tr>
<td>Fluorescein flux (nmol/cm(^2))</td>
<td>30 [10-207]</td>
<td>40 [20-246]</td>
<td>0.86</td>
</tr>
<tr>
<td>30 minutes post pH 2.0 TEER (Ω.cm(^2))</td>
<td>87 [63-110]</td>
<td>84 [71-97]</td>
<td>0.92</td>
</tr>
<tr>
<td>Fluorescein flux (nmol/cm(^2))</td>
<td>152 [22-428]*</td>
<td>249 [34-439]*</td>
<td>0.73</td>
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</table>

**Table 1** | Esophageal permeability to fluorescein is comparable in PPI responders and partial PPI responders after 30 minutes and 30 minutes after short acid exposure. No difference in TEER was observed for partial and complete PPI responders. Permeability to fluorescein is increased by exposure to luminal acid (* p=0.03 vs. permeability without exposure to luminal acid). Data are presented as median [IQR] and tested using a Mann Whitney U test.

Subsequently, we aimed to study the effect of short acid exposure on esophageal mucosa. For this purpose we replaced the luminal buffer with acidified modified Meyler buffer (pH 2.0). A five minute period of luminal acidification significantly increased esophageal permeability to fluorescein during the following 30 minutes from 37 [16-209] to 249 [31-317] nmol/cm\(^2\) (p=0.03, Signed rank test). However, no statistical difference in response to acidification in esophageal permeability and TEER was found between partial responders compared to complete responders (Table 1).

**Esophageal sensitivity**

As we hypothesized that visceral hypersensitivity is an important determinant of persistent symptoms, we determined esophageal sensitivity using infusion of neutral and weakly acid solution and balloon distension in the distal and proximal esophagus. As demonstrated in Figure 2, esophageal sensitivity for balloon distension in both the distal and proximal esophagus is significantly increased in partial PPI responders compared to complete responders (Table 2). The proximal esophagus was more sensitive to balloon distension than the distal esophagus (p<0.0001, Wilcoxon signed rank test). This was observed both in patients with a complete and partial PPI response (p=0.01 and p<0.01 respectively, Wilcoxon signed rank test).

In the distal esophagus, no significant difference was observed in reported VAS scores after a 3 minute neutral or weakly acid infusion between partial and complete PPI responders (Table 2). In the proximal esophagus reported VAS scores tended to be higher in partial responders compared to complete PPI responders, mainly for a neutral solution, but no statistical significance was reached (p=0.08, Table 2.).
Distal esophagus

Sensitivity to distension

Area under the curve

Proximal esophagus

Sensitivity to distension

Area under the curve

FIGURE 2 | Sensitivity to distension is significantly higher in partial responders compared to complete responders, both in the distal and proximal esophagus. Area under the curve values were compared using a Mann-Whitney-U test. The upper esophagus is more sensitive to balloon distension than the distal esophagus (median AUC 488 [144-757] vs. 246 [63-468], p<0.001, Wilcoxon signed rank test). This was observed for responders (p<0.01) as well as for partial responders (p<0.01).
Partial PPI responders
(n=9)  

Proximal esophagus (15 cm above LES)

<table>
<thead>
<tr>
<th></th>
<th>Partial PPI responders</th>
<th>PPI responders</th>
<th>P</th>
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<tbody>
<tr>
<td>Area under the curve of VAS scores following distension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS pH 7.0</td>
<td>640 [415-843]</td>
<td>145 [107-496]</td>
<td>0.01</td>
</tr>
<tr>
<td>VAS pH 4.0</td>
<td>29 [4-51]</td>
<td>3 [2-8]</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>26 [3-52]</td>
<td>3 [2-13]</td>
<td>0.14</td>
</tr>
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</table>

Distal esophagus (5 cm above LES)

<table>
<thead>
<tr>
<th></th>
<th>Partial PPI responders</th>
<th>PPI responders</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the curve of VAS scores following distension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS pH 7.0</td>
<td>463 [270-591]</td>
<td>65 [54-245]</td>
<td>0.003</td>
</tr>
<tr>
<td>VAS pH 4.0</td>
<td>12 [5-46]</td>
<td>3 [2-13]</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>12 [4-62]</td>
<td>4 [2-13]</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**TABLE 2 |** Esophageal sensitivity to distension is significantly higher in partial PPI responders compared to complete responders, both in the proximal and in the distal esophagus. In the distal esophagus, no significant difference were observed in reported VAS scores after a 3 minute neutral or weakly acid infusion between partial and complete PPI responders. In the proximal esophagus reported VAS scores mainly for a neutral solution tended to be higher in partial responders compared to complete PPI responders, but no statistical significance was reached. Data are presented as median [IQR] and tested using a Mann Whitney U test.

**Postprandial reflux**

The number of reflux episodes was significantly higher in partial responders compared to complete responders (13 [9.0-17] vs. 6.0 [4.0-10] p<0.01). In line with previous studies, the proximal extent was significantly higher in patients with partial response compared to patients with complete response on PPIs (11 [10-12] vs. 9.0 [7.0-10] cm p=0.05, Figure 3.). Interestingly, 8 of 9 partial PPI responders had a mean proximal extent of ≥ 10 cm, compared to only 2 of 9 complete responders (p=0.02, Fisher’s exact).

The number of acid reflux episodes and the rate of acid reflux were similarly low for partial and complete responders (1.5 [0-8] vs. 2.0 [0-3.0], p=0.89 and 8.0 [0-48] % vs. 20 [0-30] %, p=0.96). In line, no difference in the esophageal acid exposure time was observed (0.3 [0-6.7] % vs. 0.4 [0-2.3] %, p=0.67).

**Acid pocket**

We recently reported that the gastric acid pocket is a major player in the pathogenesis of GERD. Therefore we compared the pH, position and size of the acid pocket in responders and partial responders. The acid pocket was scintigraphically visible in 16 of 18 patients within 15 minutes after meal ingestion. In 2 patients (1 responder, 1 partial responder) no pooling of $^{99}$Tc-pertechnetate in the proximal stomach was observed.
As a more proximal position of the acid pocket is an important risk factor for acid reflux, we analyzed the position of the acid pocket in complete and partial responders. Also on PPIs acid reflux occurred mainly when the acid pocket was located above (17 of 35 reflux episodes [49%]) or at the level (36 of 79 [46%]) of the diaphragm, but seldom when the pocket was located below the
diaphragm (10 of 139 [7.2%]). However, no difference in position of the acid pocket was observed between partial responders versus complete responders (localisation below diaphragm: 56 ± 10% vs. 65 ± 10%, p=0.54, Figure 3.).

At the end of the measurement, the radioactive material was aspirated via the working channel of the HRM catheter. Scintigraphic activity in the proximal stomach disappeared after aspiration in both patient groups. No difference was observed in the pH of the aspirated acid pocket fluid (Figure 3.).

**Interaction of mechanisms**

To determine the interaction of mechanisms underlying PPI resistant symptoms in GERD patients we evaluated the potential interactions between the different outcome parameters. Interestingly, we found a significant correlation between both upper and lower esophageal sensitivity and the mean proximal extent of refluxate. (Figure 4.) As demonstrated in Figure 4, 8 of 9 (89%) partial PPI responders had both high proximal extent and high sensitivity, whereas 7 of 9 complete responders had a low proximal extent and a low sensitivity. Moreover, the number of reflux episodes significantly correlated with distal esophageal sensitivity to distension (r=0.53, p<0.05), demonstrating that a higher number of reflux episodes might lead to a more sensitive esophagus.

![Figure 4](image_url)

**FIGURE 4** | Patients with a partial response to PPIs had a higher proximal extent of refluxate and were hypersensitive to esophageal distension compared to patients with a complete symptomatic response after PPIs. As demonstrated in Figure 4, 8 of 9 partial PPI responders had both high proximal extent and high sensitivity, marked by the blue circle, whereas 7 of 9 complete responders had a low proximal extent and a low sensitivity (orange circle).

Previous studies in patients off PPI demonstrated a clear inverse relation of acid exposure and mucosal integrity. Therefore we correlated postprandial acid exposure and markers of mucosal integrity (i.e. flux of fluorescein and TEER). No relation was found between esophageal acid
exposure on PPIs and the basal TEER or flux. However, it should be noted that our study was performed in the postprandial period with a median acid exposure of only 0.3 [0-2.4] % of time.

**DISCUSSION**

Visceral hypersensitivity, impaired mucosal integrity and differences in the acid pocket have been hypothesized to contribute to PPI resistant symptoms in GERD patients. In the current study we demonstrated that mucosal permeability and the position of the acid pocket are similar in PPI partial responders and responders and are therefore less likely to explain persistent symptoms. In contrast, esophageal sensitivity to distension and the number of reflux episodes with a higher proximal extent are increased in PPI partial responders compared to PPI responders. This is the first study to demonstrate that PPI resistant symptoms are most likely explained by increased proximal reflux in a hypersensitive esophagus, independent of increased esophageal permeability. Clearly, this insight can be of great importance to better target these symptoms and develop more effective treatments.

Refractory symptoms to PPIs have become the most common cause for presentation of GERD patients in third referral gastrointestinal practices, and occur in approximately 30% of patients. Most patients with true GERD have at least a partial response to PPIs. Moreover, PPIs have a good safety profile, are widely available and are very effective in the treatment of reflux esophagitis. Therefore, acid suppression by means of PPIs will remain the mainstay of GERD therapy, and additional studies should mainly focus on the treatment of refractory symptoms during PPI use.

Hence, we compared the possible mechanisms underlying PPI refractory symptoms in patients with partial response to patients with PPI responders.

Previous studies in animal models and healthy volunteers have clearly demonstrated that noxious components of the refluxate such as acid, pepsin and bile acids reduce mucosal resistance and lead to impaired mucosal integrity. In the current study we indeed confirmed that mucosal permeability of esophageal biopsies of GERD patients on PPI is increased by short episodes of acid exposure. However, in contrast to previous studies, we did not find a significant difference in TEER and flux of fluorescein between partial and complete responders to PPIs. In line, Ribolsi et al. recently demonstrated that patients with PPI resistant symptoms have comparable impedance baselines, a validated measure of mucosal integrity, to patients with complete response. Based on these data we conclude that although mucosal permeability indeed increases after acid exposure, differences in mucosal permeability are unlikely to play a major role in PPI resistant symptoms.
As hypothesized by Kahrilas et al, a reflux event can trigger a chemo-sensitive or a mechano-sensitive signal from the esophagus; the first related to its acidity, the latter related to its volume. Symptoms caused by acid reflux and mediated by chemo-stimulation are very responsive to a PPI, while symptoms caused by esophageal distension and mediated by mechano-stimulation are not. In line, we here demonstrate that PPI resistant symptoms are indeed most likely triggered by mechanical stimulation of the esophagus, i.e. by reflux-induced distension of the proximal esophagus. We demonstrated that the refluxate in partial responders extended more proximal in the esophagus compared to responders, suggesting that the volume of the refluxate is an important determinant of PPI resistant symptoms. Moreover, we showed that the rate of acid reflux events and the pH and position of the acid pocket did not differ between responders and partial responders. In line, Emerenziani et al. observed that irrespective of acidity, the frequency of symptomatic reflux events in the proximal oesophagus was more than twofold that in the distal esophagus in patients with GERD. On PPI, symptoms only result from acidic reflux in a minority of patients (11%) confirming that the acidity of the refluxate is not a major determinant of PPI resistant symptoms. Based on these data, we conclude that the proximal extent or the volume of the refluxate rather than its chemical composition (i.e. acidity) is one of the main determinants of symptom perception during acid suppression.

Most interestingly, patients with PPI resistant symptoms also revealed increased sensitivity to distension, both in the proximal and distal esophagus. Of note, 89% of partial responders in our study had both reflux episodes with a high proximal extent in combination with increased sensitivity to distension, in contrast to only 11% of complete responders. The mechanisms underlying the development of the esophageal hypersensitivity to distension however remain largely unknown, but frequent exposure of the esophageal mucosa to gastric contents prior to symptoms may be involved. Clearly, reflux of larger volumes into the proximal esophagus will more likely trigger symptoms if sensitivity to distension is increased, thus explaining the origin of PPI resistant symptoms, and confirming the hypothesis that symptoms of mechanical origin are resistant to acid suppression.

Accepting that visceral hypersensitivity is an important contributory factor to persistent symptoms on PPI, one might argue that pain modulation might be beneficial in these patients. In a recent study Viazis et al compared citalopram 20 mg to placebo in 75 patients with refractory GERD symptoms on twice daily PPI. Interestingly, success rate of treatment was significantly higher in the patients receiving citalopram (62%, n=39) compared to patients receiving placebo (33%, n=36). Previous studies in patients with hypertensive esophageal disorders such as non-cardiac chest pain and esophageal spasm have clearly demonstrated that SSRIs reduce visceral hypersensitivity, and that this is associated with a reduction in symptoms. Based on the data from the current
study in combination with earlier studies, patients with refractory symptoms might possible benefit from pain modulator treatment. Additional studies to determine the effect and the mode of action are however still warranted.

A potential shortcoming of this study is the number of patients included (n=18). However, in this small population we were able perform multiple invasive measurements, enabling us to not only compare groups, but also to find interactions between the different physiological parameters. Especially as we carefully selected patients with refractory symptoms based on a positive symptom association during 24hr impedance-pHmetry, we are confident though that our results are sound and are clinically relevant.

In conclusion, we suggest that PPI resistant symptoms are most likely explained by increased proximal reflux in a hypersensitive esophagus. Hence, our data support the hypothesis that agents reducing esophageal hypersensitivity may be an effective approach to reduce PPI resistant symptoms in GERD.
REFERENCE LIST


