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Esophageal baseline impedance levels in patients with pathophysiological characteristics of functional heartburn

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Key Messages

- Analyzing a large group of patients with heartburn, normal endoscopy, and negative MII-pH, we have shown that PPI responder patients had higher AET, total reflux number, acid reflux number and proximal reflux number, lower baseline impedance values (i.e., impaired mucosal integrity) as compared with FH patients (non-responders). Moreover, we found that FH patients had a mucosal integrity comparable to HVs.
- Our data showed that negative MII-pH/PPI responders had lower PSPW values as compared with FH patients (non-responders) and that there was a direct relationship between baseline impedance levels and the PSPW index. Thus, these results underline that esophageal mucosal integrity is strictly related to the efficacy of chemical clearance and then confirm that impaired esophageal chemical clearance represents a primary mechanism in the pathogenesis of mucosal damage in GERD.
- Overall, this study demonstrated that a more-in-depth pathophysiological evaluation of MII-pH tracings by the adding of baseline impedance levels and the PSPW index, could be of help to better investigate patients with heartburn and appropriately identifying those with reflux disease and in particularly those with hypersensitive esophagus, when reflux monitoring with symptom-reflux association fails to do it.

Abstract

Background *Recently, it has been suggested that low esophageal basal impedance may reflect impaired mucosal integrity and increased acid sensitivity. We aimed to compare baseline impedance levels in patients with heartburn and pathophysiological characteristics related to functional heartburn (FH) divided into two groups on the basis of symptom relief after proton pump inhibitors (PPIs).* ***Methods*** *Patients with heartburn and negative endoscopy were treated with esomeprazole or pantoprazole 40 mg daily for 8 weeks. According to MII-pH (off therapy) analysis, patients with normal acid exposure time (AET), normal reflux number, and lack of association between symptoms and refluxes were selected; of whom 30 patients with a symptom relief higher than 50% after PPIs composed Group A, and 30 patients, matched for sex and age, without symptom relief composed Group B. A group of 20 healthy volunteers (HVs) was enrolled. For each patient and HV, we evaluated the baseline impedance levels at channel 3, during the overnight rest, at three different times.* ***Key Results*** *Group A (vs Group B) showed an increase in the following parameters: mean AET (1.4 ± 0.8% vs 0.5 ± 0.6%), mean reflux number (30.4 ± 8.7 vs 24 ± 6.9), proximal reflux number (11.1 ± 5.2 vs 8.2 ± 3.6), acid reflux number (17.9 ± 6.1 vs 10.7 ± 6.9). Baseline impedance levels*

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*were lower in Group A than in Group B and in HVs ($p < 0.001$). **Conclusions & Inferences** Evaluating baseline impedance levels in patients with heartburn and normal AET could achieve a better understanding of pathophysiology in reflux disease patients, and could improve the distinction between FH and hypersensitive esophagus.*

Keywords *esophageal sensitivity, functional heartburn, GERD/GORD, multichannel impedance and pH, PPI.*

INTRODUCTION

Combined esophageal multichannel intraluminal impedance and pH monitoring (MII-pH) is currently used for assessment of gastro-esophageal reflux disease (GERD) by measuring changes in electrical impedance caused by fluid and/or gas reflux.[1] In the absence of reflux episodes and swallows, the esophageal lumen is collapsed and the metallic rings are in close contact with the esophageal mucosa; thus the resulting baseline impedance level is determined by the intrinsic electrical conductivity of the surrounding esophageal wall.

Farre *et al.*[2] performed acid perfusion experiments in healthy subjects and demonstrated that baseline impedance level drops and maintains a low value after acidic solutions. Moreover, consistently with the finding that there was a positive correlation between *in vivo* basal impedance and *in vitro* trans-epithelial resistance values, the authors suggested that impedance baseline measurements might be used to evaluate the status of the esophageal mucosa and to study the role of the impaired mucosal integrity in acid-induced heartburn. In line with this assumption, Kessing *et al.*[3] showed that distal baseline impedance levels in GERD patients, both with pathological and physiological esophageal acid exposure time (AET), are markedly lower than those in healthy volunteers. Furthermore, a negative correlation was observed between esophageal AET and distal baseline impedance. Moreover, it has been demonstrated that baseline impedance is higher on PPI, which further suggests that baseline values are affected by acid exposure.[3]

Typical GERD symptoms (i.e., heartburn and/or regurgitation) in the presence of a normal esophageal mucosa have been used to define non-erosive reflux disease (NERD)[4] and, at the same time, are prerequisites for functional heartburn (FH). Indeed, according to Rome III criteria, the lack of correspondence between symptoms and acid reflux episodes, together with a normal acid exposure in the distal esophagus and a negative response to acid suppression, suggest a diagnosis of FH.[5,6] More recently, different studies highlighted that, to be diagnosed with FH, patients should also display a negative symptom association for non-acid reflux episodes as assessed by means of MII-pH.[7–10] On the other hand, patients showing a close temporal relationship between symptoms and acid or non-acid reflux episodes have been defined as hypersensitive esophagus (HE).[7,11,12] Recently, Woodland *et al.*[13] observed that, within both NERD and FH, patients who showed a positive acid sensitivity test had lower baseline impedance than those who did not. Of note, the authors found that a subgroup of patients with FH, despite having a normal MII-pH study and a negative response to proton pump inhibitors (PPIs), had a mucosal integrity behavior phenotype that was very similar to patients with NERD. However, it is well known that in PPI responder patients with typical reflux symptoms, a diagnosis of GERD, as defined by currently accepted reference standards, is not always confirmed.[6,14–16] On the basis of these evidences, it is not clear how to define these patients[17] and, so far, we speculated that decreased baseline impedance levels may be of additional help to distinguish patients with NERD from those with FH. Thus, the aim of this study was to compare baseline impedance levels in patients with heartburn and pathophysiological characteristics related to FH divided into two groups on the basis of symptom relief after PPIs.

MATERIALS AND METHODS

Study subjects

Throughout 2012, we prospectively enrolled consecutive endoscopy-negative patients, with typical reflux symptoms (i.e., heartburn with/without regurgitation), presenting to the outpatient esophageal pathophysiology center at the Universities of Genoa, Pisa and Padua and Hospital of Modena. The inclusion criteria were as follows: age higher than 18 years; complaining of heartburn with/without regurgitation at least twice in a week for 6 months in the previous year. The exclusion criteria were as follows: pregnancy (excluded by urine analysis) and/or breast feeding; eating disorders; history of thoracic, esophageal, or gastric surgery; primary or secondary severe esophageal motility disorders; underlying psychiatric illness; use of non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin; peptic ulcer at a previous endoscopy. All patients signed an informed consent. The study was designed and carried out in accordance with the Helsinki Declaration (Sixth revision, Seoul 2008) approved by the institutional review boards.

The presence of erosive esophagitis and other abnormalities was excluded by means of an upper endoscopy performed in the above mentioned Divisions of Gastroenterology during the 6 months prior to the visit. Each patient discontinued PPIs or H2-receptor antagonists at least 20 days before undergoing the endoscopy. A distinct investigator completed a structured

interview to the patients, including a careful medical history (with recording of height and weight), current medications, tobacco use, and alcohol consumption.

Following the first visit, single standard dose of esomeprazole or pantoprazole therapy was prescribed to each patient for 8 weeks. Symptoms were evaluated both before and after 8 weeks of PPI therapy through a validated questionnaire (GERD Impact Scale: GIS)[18] and a visual analog scale (VAS) for heartburn. GERD Impact Scale comprises eight questions about the frequency, over the previous 2 weeks, of the following items: acid-related symptoms; chest pain; extra-esophageal symptoms; impact of symptoms on sleep, work, meals, and social occasions; use of additional non-prescription medications. Four response options were allowed to describe the frequency of the above items over the previous 2 weeks: 'none of the time',[1] 'a little of the time',[2] 'some of the time'[3], and 'all of the time'.[4] Patients were also asked to rate their satisfaction with symptom control on a global VAS from 0 (no relief at all) to 100 (complete symptom relief). The VAS score has been used as a self-assessment tool for symptom measure, which has been adopted in many other trials for evaluation of visceral symptoms.[19,20]

All the subjects underwent stationary esophageal manometry and 24-hour MII-pH esophageal monitoring off therapy (14-day washout). Indeed, patients who reported a satisfactory symptom relief after PPIs were considering antireflux surgery, and patients with an unsatisfactory symptom relief underwent the exams to investigate the underlying possible causes. Patients were only allowed to take alginates, on as-needed basis, as rescue therapy for controlling heartburn.[21,22] Stationary manometry and MII-pH were performed after an overnight fast.

A group of 20 healthy volunteers (HVs), who never experienced gastrointestinal symptoms, underwent esophageal manometry and MII-pH.

Stationary esophageal manometry

All subjects underwent stationary esophageal manometry to determine the distance of the proximal border of the lower esophageal sphincter (LES) from nostrils and to exclude the presence of abnormal peristalsis. This study was performed by means of an eight-channel water-perfused manometric catheter with an external diameter of 4.5 mm (Dyno 2000® Menfis; BioMedica, Bologna, Italy), equipped with a computer-based data recording and storing. Esophageal body motility and LES relaxation were tested by at least 10 wet swallows of 5 mL of water. Wave amplitude and duration were measured by means of four openings located 5, 10, 15, and 20 cm above the LES, respectively. A station pull-through technique was then used to accurately locate the position of LES.

Esophageal multichannel intraluminal impedance and pH monitoring

MII-pH was performed using a polyvinyl catheter (diameter: 2.3 mm), equipped with an antimony pH electrode and several cylindrical electrodes, with a length of 4 mm, placed at intervals of about 2 cm (Sandhill Scientific Inc., Highland Ranch, CO, USA). Each pair of adjacent electrodes represented an impedance-measuring segment corresponding to one recording channel. The single-use MII-pH catheter was positioned with the pH electrode 5 cm above the LES and the six impedance recording channels at 3, 5, 7, 9, 15, and 17 cm above the LES. All patients consumed foods and beverages exclusively during three standard meals (lunch at 1.00 pm, dinner at 8.00 pm, and breakfast at 8.00 am of the next day) on the basis of a Mediterranean diet,[23] without alcohol and coffee, to reduce variability due to alimentary habits. They were instructed to indicate the beginning and ending times of meals. The patients were also requested to remain in upright position during the day and to indicate the recumbent period during nighttime (max 8 h). Each patient was instructed to press the 'event marker' button, on the pH datalogger, whenever they experienced reflux symptoms during the recording period.

MII-pH data analysis

At the end of the recording period, MII-pH tracings were reviewed manually by two investigators (NdB, ES) blinded for the conditions of the patients to ensure accurate detection and classification of reflux episodes and baseline impedance values. Meal periods were excluded from the analysis. Impedance and pH data were used to determine the number and type of reflux episodes and AET (reflux percent time) in each patient. In particular, distal esophageal AET was defined as the total time with pH below four, divided by the total time of monitoring. A percent time lower than 4.2% with pH <4, over 24 h, was considered as normal.[7,23]

Acid, weakly acidic, and weakly alkaline refluxes were defined according to the literature.[24] Proximal reflux extent was defined as a drop in impedance recorded at 15 cm from LES. Finally, correlation between symptom and reflux events with symptom index and symptom association probability (SI and SAP) was evaluated for each patient as previously described.[25] Baseline impedance levels were assessed from the most distal channel (*z3*, 3 cm above the LES) during the overnight rest. In a subgroup of 20 consecutive patients, the appraisal of baseline impedance values was performed for 6 h during night times, excluding reflux episodes, swallows, and pH drops. These baseline values were then compared with a short-time evaluation performed at three time points (around 1.00, 2.00, and 3.00 am). In particular, we selected 10 min around each time point avoiding swallows, refluxes, and pH drops. Considering the almost perfect concordance between short- and long-time analyses (as detailed in the result section), we performed only a short-time analysis for all the remaining patients. Moreover, for each patient, a novel parameter representing chemical clearance namely the postreflux swallow-induced peristaltic wave (PSPW) index was measured.[26]

According to endoscopy and MII-pH data analysis, patients were included into the study in case of normal endoscopy, normal AET, normal number of reflux episodes, and lack of association between symptoms and refluxes. Therefore, we evaluated in them the symptom relief after PPI therapy using GIS and VAS scores and we further stratified these patients into two groups by means of therapeutic outcome as follows: 'Group A' consisting of 30 patients who reported a satisfactory symptom relief for heartburn (>50% compared to baseline values); 'Group B' (patients with FH)[27] consisting of an equivalent number of patients, matched for sex and age, who reported an unsatisfactory symptom relief for heartburn (<50% compared to baseline values).

Finally, MII-pH parameters, including AET, number of refluxes (acid and non-acid), and baseline impedance values, were collected from HVs and compared with those collected from Group A and Group B.

Statistical analysis

Data are expressed as mean and SD. Statistical tests to compare groups of subjects included Student's *t*-test and ANOVA for difference in mean values, Mann–Whitney *U* and Kruskal–Wallis tests for skewed variables, Pearson's Chi-squared test (with

Yates's continuity correction as appropriated) for difference in counts and frequency. *Post hoc* comparisons were performed using the Bonferroni correction in case of a significant ANOVA result. The Kolmogorov–Smirnov test was used to assess the normality of data. A $p < 0.05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics

Sixty patients were included in the study and further divided into two distinct groups as previously reported. The baseline characteristics of these individuals have been shown in Table 1. Patients in Group A (10 M and 20 F, mean age 49.7 ± 12.4 years) and in Group B (10 M and 20 F, mean age 50.3 ± 11.5 years) had no significant differences in age, gender, and body mass index (p = ns). Healthy volunteers (10 M and 10 F, mean age 49.9 ± 14.7) also had no differences in mean age and mean body mass index. Seven of 60 patients (11.7%) were regular smokers, 16/60 (26.7%) were used to take at least a cup of coffee daily, and 5/60 (8.4%) reported 2-to-3 units of alcohol consumption per day. We did not record any differences in behaviors between patients and HVs. The prevalence of hiatal hernia did not differ between Group A (12/30, 40%) and Group B (10/30, 33.4%; $p = 0.184$). The prevalence of hiatal hernia was higher in both groups, A and B, as compared with HVs ($p < 0.001$).

As to the symptom relief after PPI therapy, within Group A, the mean value of GIS decreased from 1.77 before therapy to 0.36 after therapy ($p < 0.05$). By contrast, the GIS score within Group B remained unmodified ranging from 1.78 to 1.53. The VAS questionnaire, indicating heartburn perception, changed in Group A from 93.4 to 18.9 ($p < 0.05$), but no significant modifications were found in Group B in which the perception changed from 91.7 to 68.3. Overall, the mean symptom relief after PPI therapy was 74.5 ± 8.6% in Group A *vs* 23.6 ± 10.6% in Group B ($p < 0.001$).

Pathophysiological investigations

The selected endoscopy-negative patients did not present major abnormal motility related disorder at the stationary manometry evaluation and their LES tone values fell within the physiological range in all patients. Combined MII-pH was well-tolerated by all subjects and no technical failures occurred. The results yielded by MII-pH testing are displayed in Table 2. All patients had negative MII-pH test that was physiological AET, normal reflux number, and lack of association between symptoms and refluxes. The mean number of heartburn episodes reported during the 24-h MII-pH test was 3.7 in Group A and 3.3 in Group B.

Of note, Group A (*vs* Group B) showed a mild increase in the following parameters: mean AET (1.4 ± 0.8% *vs* 0.5 ± 0.6%; $p < 0.001$), mean reflux episodes (30.4 ± 8.7 *vs* 24 ± 6.9; $p = 0.003$), mean proximal reflux events (11.1 ± 5.2 *vs* 8.2 ± 3.6; $p = 0.024$), and acid reflux episodes (17.9 ± 6.1 *vs* 10.7 ± 6.9; $p < 0.001$). Similarly, Group A showed higher MII-pH values as compared with HVs: mean AET (1.4 ± 0.8% *vs* 0.7 ± 0.6%; $p < 0.001$), mean reflux episodes (30.4 ± 8.7 *vs* 17.9 ± 10.8; $p < 0.001$), and acid reflux episodes (17.9 ± 6.1 *vs* 11.7 ± 8.4; $p < 0.001$). No differences were found between Group B and HVs in reflux monitoring parameters (AET, acid and non-acid refluxes) with the exception for mean reflux episodes (24 ± 6.9 *vs* 17.9 ± 10.8; $p = 0.02$). All detailed results are reported in Table 2.

Comparing mean baseline impedance values collected in a short-time period (10 min), three times during the night, with those during a long-time period (6 h), we obtained a great interclass correlation (ICC = 0.99) between the two methods (Fig. 1). Thus, suggesting that baseline values remain stable during

Table 1 Epidemiological characteristics of Group A and B

	Group A (30 pts)	Group B (30 pts)	HVs	*p*
Male/Female	10/20	10/20	10/10	n.s.
Mean age (SD)	49.7 ± 12.4	50.3 ± 11.5	49.9 ± 14.7	n.s.
BMI	24.2 ± 3.4	24 ± 3.7	24.1 ± 3.5	n.s
Smokers	4 (13.3%)	3 (10%)	3 (15%)	n.s.
Alcohol (2–3 U/die)	3 (10%)	2 (6.7%)	2 (10%)	n.s.
Coffee (2 cups/die)	8 (26.7%)	8 (26.7%)	5 (25%)	n.s.
Hiatal hernia	12 (40%)	10 (33.4%)	0	<0.001

Table 2 Pathophysiological characteristics of Group A and B

	Group A (30 pts)	Group B (30 pts)	HVs	*p*
AET	1.4 ± 0.8*	0.5 ± 0.6	0.7 ± 0.6	<0.001
Total number of refluxes	30.4 ± 8.7*	24 ± 6.9*	17.9 ± 10.8	0.003
Proximal refluxes	11.1 ± 5.2	8.2 ± 3.6	9.5 ± 9.5	0.024
Acid refluxes	17.9 ± 6.1*	10.7 ± 6.9	11.7 ± 8.4	<0.001
Non-acid refluxes	12.5 ± 5.1	13.3 ± 5.5	12.7 ± 6.2	0.545
Gas refluxes	13.6 ± 10.7*	10.3 ± 5.9	9.9 ± 6.8	0.145
PSPW index	56.2 ± 8.8*	71.1 ± 6.1	76.1 ± 13	<0.001

*$p < 0.05$ *vs* HVs.

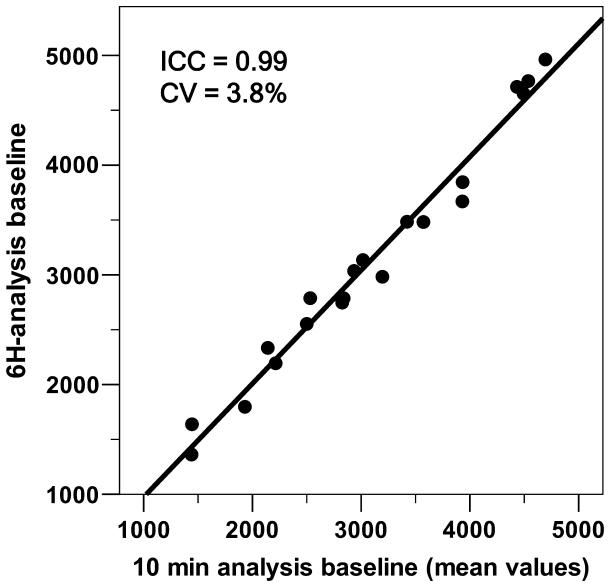

Figure 1 Correlation between short-time (10 min) periods of baseline impedance measurements, for three time points around 1, 2, and 3 am, compared with long-time (6 h) periods, during night times ($p = 0.24$).

nighttime, and when recorded for short periods they are reliably representative of a larger time analysis window.

Baseline impedance levels were lower in Group A (at 1 am 2071.5 ± 713; at 2 am 2203.1 ± 753.2; at 3 am 2234.4 ± 668.8) than in Group B (at 1 am 3634.3 ± 1117.1; at 2 am 3850.3 ± 954.5; at 3 am 3862.1 ± 840.9) and HVs (at 1 am 3317.7 ± 953; at 2 am 3443.4 ± 950.1; at 3 am 3522.6 ± 915.6) at all the three time points ($p < 0.05$), as shown in Fig. 2. No differences were found between Group B and HVs. Moreover, a negative correlation between esophageal AET and baseline impedance levels of both groups was found ($r = -0.558$; $p < 0.001$; Fig. 3).

The PSPW index was lower in Group A than in Group B (56.2 ± 8.8% *vs* 71.1 ± 6.1%; $p < 0.001$). A positive correlation was observed between the PSPW index and baseline impedance levels of both groups ($r = 0.623$; $p < 0.001$; Fig. 4).

DISCUSSION

Our study specifically aimed to determine whether there was a difference in baseline impedance levels, which indicate the presence of an impaired mucosal integrity, between patients with negative MII-pH test and a positive or negative response to PPI. Moreover, as secondary aim, we evaluated in the same subgroups of patients the PSPW index, which represents the efficacy of esophageal chemical clearance, to correlate it with

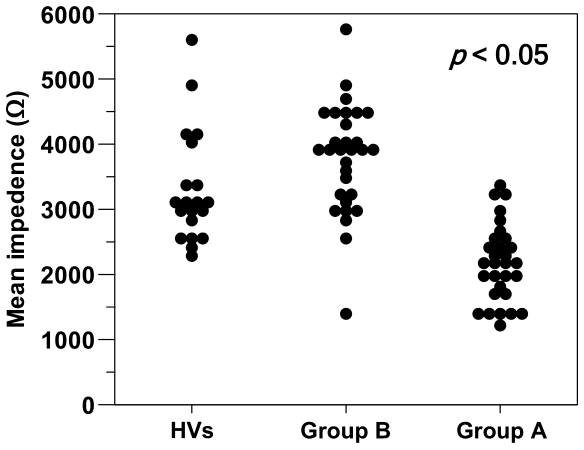

Figure 2 Difference in baseline impedance values between Group A, Group B ,and HVs (mean values) during night times.

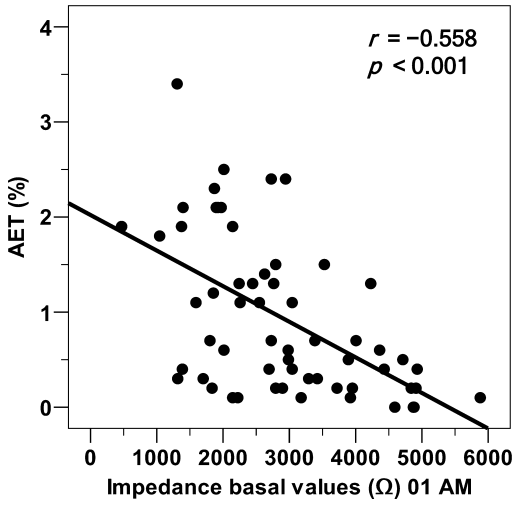

Figure 3 Linear correlation between acid exposure time and baseline impedance values.

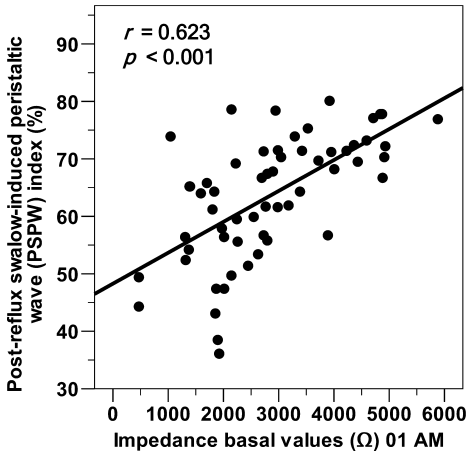

Figure 4 Linear correlation between postreflux swallow-induced peristaltic wave index and basal impedance values.

the different degree of mucosal integrity as expressed by baseline impedance levels.

Analyzing a large group of patients with heartburn, normal endoscopy, and negative MII-pH, we have shown that: (i) PPI responder patients (Group A) had higher AET, total reflux number, acid reflux number, and proximal reflux number as compared with FH patients (Group B); (ii) PPI responder patients (Group A) had lower baseline impedance levels and PSPW values as compared with FH patients (Group B); (iii) there was a direct relationship between baseline impedance levels and the PSPW index and an inverse relationship between baseline impedance levels and AET; (iv) FH patients showed similar baseline impedance values and PSPW index to HVs.

This is the first study that closely considers patients with heartburn, negative endoscopy, and negative pathophysiological examinations, with different symptom relief after PPI therapy. By means of baseline impedance values and PSPW index, this study highlights the differences between patients with different response to PPI therapy, whereas symptom association parameters (SI and SAP) fail to do it. In this study, baseline impedance levels have been assessed during nighttime as there is a greater facility to avoid swallows and refluxes, which usually occur in small number during the night. In addition, Kessing *et al.*[3] performed 24-hour measurement of baseline levels in GERD patients without finding a clear diurnal trend in such values, whereas AET decreased in all patients during the night. We calculated baseline impedance levels in the distal esophagus only. Indeed, it has been shown that proximal baseline impedance levels were higher than distal ones in GERD patients, although no significant differences in proximal baseline impedance levels have been found between GERD patients and healthy controls.[2,3,28]

The question, whether acid-induced alterations of mucosal integrity might play a role in heartburn perception in negative MII-pH/PPI responder patients, has never been investigated. We have shown that PPI responders with a negative MII-pH test have significantly higher AET and number of acid refluxes, and lower baseline impedance levels as compared with FH patients. These data, in keeping with previous findings,[3,13,29,30] suggest a possible role of acid, although at physiological levels, both in heartburn perception and in response to acid suppression. In line with the above mentioned studies, we also observed a negative correlation between AET and esophageal distal baseline impedance. The mechanisms responsible for reflux perception are not yet fully understood. In this regard, acid reflux remains the most important determinant in the generation of symptoms, although MII-pH studies have shown that both acid and non-acid reflux can generate symptoms.[25,31–33] According to recent studies, activated pepsins may represent a relevant pathogenetic factor both in acid and in weakly acidic refluxes,[34–36] as pepsins activation occurs at pH <4.5, but their proteolytic activity is maintained up to pH 5.5 and denaturation occurs at pH >7.[37] In addition, duodeno-gastro-esophageal reflux is also implicated in the development of mucosal damage due to the toxic effects of components such as bile acids and pancreatic enzymes.[36] Apart from mucosal erosion, the overall pathogenetic factors are likely to impair mucosal integrity, to cause dilated intercellular spaces (DIS), and to increase mucosal permeability even in the absence of macroscopic mucosal injury.[38]

Overall, it is reasonable to hypothesize that baseline impedance levels increase in parallel with the severity of GERD, from healthy and FH subjects to HE, NERD, and erosive reflux disease (ERD). Accordingly to Ribolsi *et al.*,[30] baseline impedance levels could prove useful to increase the diagnostic sensitivity of MII-pH monitoring.

Recently in a large group of PPI-responsive patients (>75% of symptoms relief after 8 weeks of acid suppressive therapy), we did not find pathophysiological characteristics of GERD in 19% of cases.[39] We defined these patients as MII-pH negative PPI responders and attributed their positive therapeutic outcome to a placebo effect rather than to intrinsic limitations of the MII-pH technique in correlating symptoms to reflux events. On the other hand, our results in this study are in keeping with the view that low baseline impedance values could explain different therapeutic outcomes observed in patients with physiological levels of acid exposure and negative symptom association.

Another important finding of this study regards the PSPW index. After a reflux episode, esophageal clearance depends on volume and chemical clearance. Volume clearance consists of a secondary peristaltic wave, which removes around 90% of the refluxate, but neutralization of the distal esophageal lumen occurs only after transport of saliva by a swallow-induced peristaltic wave. As intraluminal impedance recording can accurately predict bolus transit, the efficiency of esophageal chemical clearance mechanisms can be assessed in the clinical setting by impedance monitoring, which allows to assess swallow-induced peristaltic waves following reflux episodes throughout a 24-hour period off and on PPI therapy. It has been shown that impairment of esophageal chemical clearance represents a primary pathophysiological mechanism

specific to GERD as it is unaffected by medical or surgical therapy and is not found in FH.[26] Moreover, it appears to have a major role in the development and persistence of esophageal mucosal damage in GERD and a high diagnostic accuracy in distinguishing GERD from non-GERD subjects as evaluated on- or off-PPI therapy.[26] Indeed, Frazzoni *et al.*[26] demonstrated that ERD patients had lower PSPW index than NERD patients. In this study, PPI responder patients with a negative MII-pH have shown a lower PSPW index than FH patients. Furthermore, a positive correlation has been observed between baseline impedance values and the PSPW index. These results show that esophageal mucosal integrity is related to the efficacy of chemical clearance. Impairment of esophageal chemical clearance is then confirmed to represent a primary mechanism in the pathogenesis of mucosal damage in GERD.

Several studies demonstrated that acid can induce esophageal DIS, which are more common in patients with increased AET, and which are decreased by PPI therapy.[40–43] Farre *et al.*[2] showed that prolonged exposure of the rabbit esophagus to luminal acid affects mucosal integrity, resulting structurally in increased size of the intercellular spaces. In GERD patients, both with pathological and with physiological AET, Zhong *et al.*[29] observed a significant negative correlation between esophageal intercellular spaces and baseline impedance. In NERD, sensitization of esophageal sensory nerve endings might be facilitated by DIS, leading to increased paracellular permeability and facilitation of acid exposure.[44,45] On the other hand, patients with FH have no DIS.[42,43,46] The question, whether patients with negative MII-pH and positive response to PPI have DIS, has not yet been investigated. Acid is also able to determine change in paracellular permeability of the esophageal epithelium via disruption of normal tight-junction morphology.[47–49] Of note, in GERD patients, both with pathological and with physiological AET, Zhong *et al.*[29] observed a significant negative correlation between baseline impedance and expression of claudin-1. However, the mechanism through which claudin-1 may reduce esophageal mucosa integrity is yet unknown. Future studies evaluating the morphological and/or molecular changes in the esophageal wall of patients with GERD and FH are warranted to determine the extent to which baseline impedance reflects esophageal structural changes.

We acknowledged the possibility that some patients with heartburn may have been misclassified owing to the MII-pH limitations,[50] and some patients could have had a good response due to a placebo effect that our study missed to consider. In particular, there is uncertainty about the validity of SI or SAP for determining the correlation between symptoms and refluxes in patients with GERD refractory to PPI therapy.[51] Indeed, in a recent study, Slaughter *et al.*[52] concluded that the likelihood of abnormal SI and SAP, in patients with symptoms refractory to PPI therapy, is low and that, at low reflux rates (i.e., 2-minute period considered as reflux positive when pH fell below 4 and lasted 5 s or more), they suffer from significant day-to-day variability in the test and cannot be relied upon with confidence. In addition, in a recent study, it has been claimed that off-PPI MII-pH findings do not predict response to PPIs in patients with typical reflux symptoms.[53]

According to our results, we believe that a more-in-depth pathophysiological evaluation of MII-pH tracings by the adding of new quantitative variables, such as baseline impedance levels and the PSPW index, could be of help to better investigate patients with heartburn and to better identify patients with reflux disease. Indeed, we believe that patients with heartburn, normal endoscopy, and negative MII-pH responding to PPIs and who have impaired mucosal integrity based on baseline impedance levels should be considered as NERD, and particularly as affected by HE. In this regard, it is reasonable to assert that both baseline impedance values and PSPW index might be helpful to confirm GERD diagnosis in endoscopy-negative PPI responders, who are willing to undergo surgical or endoscopic therapies, in which all traditional MII-pH variables (i.e., AET, reflux number), assessed off PPI, result in the normal range but who did not refer any symptoms during the 24-hour recording time.

In conclusion, to the best of our knowledge, this is the first study evaluating new variables of MII-pH study (baseline impedance levels and the PSPW index) in patients with heartburn and negative MII-pH, but positive response to PPIs. Based on our data, in these patients, baseline impedance levels are related to the chemical clearance of their esophagus and concomitant with physiological levels of esophageal acid exposure. Moreover, considering that lower baseline impedance levels have been found in patients with negative MII-pH and positive PPI response compared to patients with negative MII-pH and negative PPI response, we believe that the assessment of esophageal baseline impedance could represent a marker for acid reflux-induced changes to the esophageal mucosa and may help to identify patients affected by hypersensitive esophagus, especially when reflux-symptom association analysis fails to do it. However, the results from this study warrant further research in humans to

validate the measure of baseline impedance values, also comparing such novel parameters with the gold standard technique.

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CONFLICTS OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

IM, NdB, ES designed the research study; performed the research; collected and analyzed the data; wrote the article; PP, AA contributed to the design of the study; performed statistical analysis; wrote the article; MB, VS, MF, SM contributed to the design of the study; wrote the article; All authors approved the final version of the manuscript.

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