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Impedance-pH reflux patterns can differentiate non-erosive reflux disease from functional heartburn patients

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Abstract

Introduction Symptom association is important to distinguish non-erosive reflux disease [NERD; abnormal oesophageal acid exposure time (AET) and/or positive symptom association] from functional heartburn (FH; normal AET and negative symptom association). Asymptomatic patients during reflux monitoring are challenging as symptom association cannot be assessed.

Aim To evaluate whether impedance-pH reflux patterns are useful to differentiate NERD from FH.

Methods Endoscopy-negative reflux patients underwent impedance-pH off-therapy. Oesophageal AET, characteristics of reflux episodes and symptom association probability (SAP) were measured. Twenty patients asymptomatic during the first test repeated a second examination.

Results Of 329 patients, 130 (40%) were pH-POS, 120 (36%) pH-NEG/SAP+ (hypersensitive oesophagus = HO) and 79 (24%) pH-NEG/SAP− (FH). Total and acid reflux episodes were significantly higher ($p < 0.01$) in pH-POS compared to pH-NEG/SAP+, pH-NEG/SAP− and healthy volunteers (HVs). Patients pH-NEG/SAP+ had a significantly increased number of weakly acidic reflux episodes compared to pH-POS, pH-NEG/SAP− and HVs ($p < 0.01$). The rate of proximal reflux episodes in pH-POS (50%) and pH-NEG/SAP+ (47%) was higher ($p < 0.01$) than in pH-NEG/SAP− (33%) and HVs (33%). Measuring AET, number of reflux episodes and percentage of proximal reflux events permits to identify FH in 70% of cases and HO in 80% of cases who repeated the examination.

Conclusion In patients with normal AET and SAP+, increased number of weakly acidic reflux and higher rate of proximal reflux are the main discriminant features. There is large overlap between FH and HVs. These differences can be of help in diagnosing patients with normal oesophageal acid exposure who fail to have symptoms during MII-pH testing.

Keywords Impedance pH-metry · NERD · Hypersensitive oesophagus · Functional heartburn

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Introduction

Gastro-oesophageal reflux disease (GERD) is one of the most common gastrointestinal diseases in western countries [1]. Recent studies documented that up to 70% of reflux patients have typical reflux symptoms in the absence of endoscopically visible oesophageal mucosal injuries, making non-erosive reflux disease (NERD) the most common form of GERD [2].

Endoscopy-negative patients incorporate subgroups which differ significantly from a pathophysiological point of view and we have proposed subclassifying them [3] on the basis of oesophageal 24-h multichannel intraluminal impedance combined with pH metry (MII-pH) results and symptom association analysis (SAP), as follows: (a) pH-POS NERD patients with abnormal distal oesophageal acid exposure, (b) hypersensitive oesophagus (HO)—patients with normal distal oesophageal acid exposure and positive symptom association for either acid and/or non-acid reflux and (c) functional heartburn (FH)—patients with normal distal oesophageal acid exposure and negative symptom association. To test this new sub-classification we have also assessed in a more recent study [4], the distribution of reflux and dyspeptic symptoms in a large cohort of NERD patients subdivided as above, and we have observed that patients with FH present more frequently functional dyspepsia (FD) than the other subsets, once again supporting the concept that impedance testing could be able to differentiate NERD patients from those with FH and that the latter ones must be definitively included in the overall population with functional GI disorders, in agreement with Rome III criteria [5].

The possibility to differentiate patients suffering of FH from the rest of NERD population represents important new information provided by MII-pH. This sub-classification is clinically important as patients of the latter group respond better to acid suppressive therapy, while those with FH have a scant response to it and have more likely an accompanying psychopathology [6]. Moreover, recent studies documented that NERD patients with normal acid exposure but positive symptom association may benefit from anti-reflux surgery procedures [7, 8]. However, it is worth nothing that some patients (5–30%) may not complain of reflux symptoms during the MII-pH monitoring and, in few cases, may also forget to press the event marker button on the MII-pH datalogger whenever they experience a symptom during the testing day [4, 9, 10]. In these cases, the above stratification based on combined MII-pH and SAP may be difficult to obtain.

We have hypothesized that impedance-pH reflux patterns differ between the two subsets of NERD and FH and this characterization may be useful to distinguish them also when symptoms do not occur or are not reported during MII-pH testing.

We carried out this study to compare the characteristics of reflux episodes in a series of endoscopy-negative patients subclassified using MII-pH monitoring in order to observe whether there are differences which allow us to separate patients with FH from the other two subsets of NERD. In addition, we repeated a second examination in a subgroup of patients who did not experience symptoms during the first testing day in order to assess the degree of concordance between the two tests and to verify whether patients with FH and NERD with HO can be differentiated on the sole basis of reflux patterns.

Methods

Subjects

We reviewed our prospectively collected data on patients with typical GERD symptoms (e.g., heartburn and regurgitation) lasting for more than 6 months and occurring at least three times weekly, presenting consecutively to our motility centre at the University Hospital of Genoa, Italy. Exclusion criteria were: history of thoracic, oesophageal, or gastric surgery; primary or secondary severe oesophageal motility disorders; evidence of erosive oesophagitis at previous (2–5 years) endoscopy; presence of dyspeptic symptoms as major disturbances. Patients were asked to discontinue any medication that would influence oesophageal motor function at least 1 week before performing tests of oesophageal function.

The study protocol was approved by the local ethics committees and performed according to the Declaration of Helsinki. All patients provided written informed consent to use collected data before their data were used in this study.

Oesophageal impedance and pH monitoring

Oesophageal impedance-pH monitoring was performed using an ambulatory multi-channel intraluminal impedance and pH monitoring system (Sleuth®, Sandhill Scientific, Inc., Highland Ranch, CO, USA). The system included a portable data logger with impedance-pH amplifiers and a catheter with one antimony pH electrode and eight impedance electrodes at 2, 4, 6, 8, 10, 14, 16, and 18 cm from the tip of the catheter. Each pair of adjacent electrodes represented an impedance-measuring segment corresponding to one recording channel. The six impedance and one pH signals were recorded at 50 Hz on a 128 MB CompactFlash (SanDisk, Milpitas, CA, USA).

Study protocol

All subjects underwent careful history taking and clinical examination and upper gastrointestinal endoscopy to assess the presence of oesophageal mucosal injury. Patients treated with antisecretory drugs were asked to discontinue acid suppressive therapy at least 30 days before the endoscopic examination. During the washout period, patients were allowed to use an oral antacid or alginate on as needed basis for the relief of heartburn. Based on the results of upper endoscopy, patients with NERD were

classified on the basis of Montreal classification and then were included in this investigation [11]. Within 1–5 days (median 3 days) from the upper endoscopy, all endoscopy-negative patients underwent MII-pH monitoring. They also underwent a stationary oesophageal manometry to assess oesophageal motor function and to locate the lower oesophageal sphincter (LOS).

The methodology of probe calibration, catheter placement, patient instruction and performance have been previously described [3]. On the monitoring day, each subject ate three standard meals of a Mediterranean diet, as previously reported [12].

All patients who did not report symptoms during the testing day and who had a normal acid exposure time (AET) at MII-pH studies were invited to undergo a second impedance-pH examination still off medication after 4 weeks in order to assess whether their reflux patterns allowed us to identify the subgroups of NERD with HO or FH they belonged, independently of SAP.

Data analysis

The data stored on the CompactFlash card were downloaded into a personal computer and analyzed using a semiautomated reflux detection algorithm (Autoscan, Sandhill Scientific, Inc.). Accuracy of reflux detection was verified manually by an expert reader (ES).

Definitions of reflux episodes

Liquid reflux was defined as a retrograde 50% drop in impedance starting distally (at the level of the LOS) and propagating to at least the next two more proximal impedance measuring segments. Gas reflux was defined as a rapid (3 kΩ/s) increase in impedance >5000 Ω, occurring simultaneously in at least two oesophageal measuring segments, in the absence of swallowing. Mixed liquid–gas reflux was defined as gas reflux occurring immediately before or during a liquid reflux.

Simultaneously recorded pH data were used to classify reflux episodes as acid, weakly acidic, or weakly alkaline according to the previously reported criteria [13].

Gastro-oesophageal reflux parameters

Impedance and pH data was used to define: number and type of reflux episodes, acid exposure [refluxate presence time (minutes) and refluxate percent time], mean acid clearance time, volume clearance time, proximal extent (number and percent of reflux episodes reaching 15-cm above LOS). Parameters were reported separately for upright and recumbent periods. Meals were excluded from the analysis.

Total 24-h oesophageal AET was defined as the total time at pH below 4 divided by the time of monitoring and a value lower than 4.2% over 24 h was considered normal [12, 14].

For comparisons, normal values were obtained from 48 healthy volunteers (HVs) studied in ambulatory conditions eating the same standardized meals. The 95th percentile was considered to be the upper limit of normal values in this series.

Symptom-reflux association analysis

In each patient we calculated the SAP for typical oesophageal symptoms. In the analysis we separated symptoms associated with acid reflux from those associated with non-acid reflux (including weakly acidic and weakly alkaline reflux as a whole) and symptoms occurring independently of reflux episodes.

The SAP was calculated for both acid and non-acid reflux using a custom made Excel macro function (RT) using the algorithm described and validated by Bredenoord et al. [15] and was considered positive if >95%.

Statistical analysis

Differences in proportions were compared using the chi-square or Fisher's exact test, depending on the sample size. Unless otherwise specified, data are presented as median and percentile values (25th, 75th, 95th percentile). Since data were not normally distributed, differences between groups were compared using Kruskal–Wallis and/or Mann–Whitney tests. Spearman's rank correlation test was used to compare the diagnosis of HO/FH based on impedance-pH parameters only and that based on impedance-pH results combined with SAP. The sensitivity, specificity, positive and negative predictive value and accuracy were calculated. Differences were considered statistically significant when $p < 0.05$.

Results

After upper endoscopy, 369 patients were identified as endoscopy-negative. During the monitoring period, 329 of them reported at least one type of typical GERD symptom and were included in the initial analysis.

Detailed demographic data of our patients are shown in Table 1. The median body mass index (BMI) of endoscopy-negative patients did not differ between male (25 kg/m^2, range 18–42 kg/m^2) and female (23 kg/m^2,

Table 1 Demographic and clinical characteristics of endoscopy-negative patients ($n = 329$)

Demographic and clinical parameters	pH-POS	pH-NEG/SAP+	pH-NEG/SAP−	p value
Patients (n)	130	120	79	
Female/male patients (n)	58F/72M	71F/49M	55F/24M	<0.01
Mean age (years)	51 (20–84)	45 (20–77)	48 (18–76)	NS
Mean BMI	27 (18–45)	24 (16–48)	23 (16–34)	<0.01
Alcohol consumption (%)	47.7	35.8	32.9	NS
Coffee consumption (%)	78.5	69.2	74.7	NS
Tobacco use (%)	20.0	20.8	25.3	NS
Prevalence of hiatal hernia (%)	71.5	38.3	35.4	<0.01
Patients having previously received PPIs [n (%)]	86 (66)	114 (95)	77 (97)	<0.01
Positive (≥50%) symptom response [n (%)]	68 (79)	58 (51)	15 (19)	<0.01

Fig. 1 Median values of oesophageal acid exposure time (AET) in HVs ($n = 48$) and in pH-POS patients ($n = 130$), pH-NEG/SAP+ patients ($n = 120$) and pH-NEG/SAP− patients ($n = 79$). *$p < 0.05$ versus HVs; §$p < 0.05$ versus pH-NEG/SAP− patients; #$p < 0.05$ versus pH-NEG/SAP+ patients
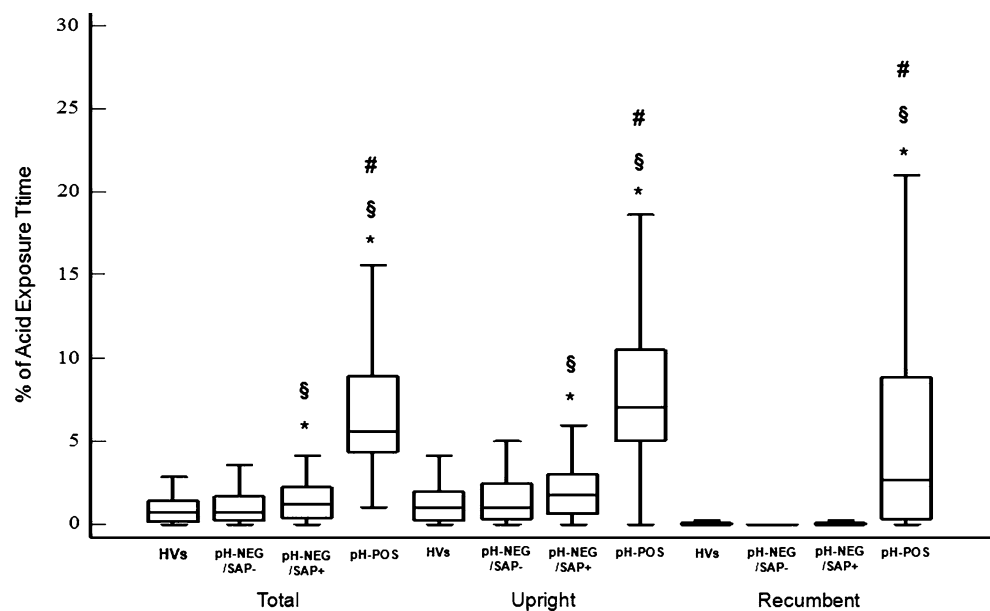
range 16–41 kg/m^2) patients. HVs (27F; mean age 44 years; range 22–77 years) had similar demographic and BMI (23 kg/m^2, range 16–34 kg/m^2) characteristics compared to reflux patients.

Acid exposure and symptom-reflux association

The total number of symptoms reported by endoscopy-negative patients was 2861 (mean 11.7, range 2–131). Patients reported 2367 heartburn events (mean 9, range 1–92) and 1494 regurgitation episodes (mean 9, range 1–99). We found an abnormal distal oesophageal AET in 130 (40%) patients (pH-POS subgroup) and among the 199 patients (60%) with normal acid in their oesophagus, 120 (36%) had a positive SAP (pH-NEG/SAP+; HO subgroup). The remaining 79 (24%) patients with normal acid

and no association between symptoms and any type of reflux were pH-NEG/SAP− (FH subgroup).

pH-metry data

Patients pH-POS had significantly longer distal oesophageal AET compared to pH-NEG/SAP+, pH-NEG/SAP− and HVs [5.6 (4.4–8.9; 17.3) vs. 1.2 (0.4–2.2; 3.3) vs. 0.7 (0.2–1.7; 2.9) vs. 0.7 (0.2–1.4; 4.2); respectively ($p < 0.01$)]. This was true in both upright and recumbent body position ($p < 0.01$). Moreover, distal oesophageal AET was greater ($p < 0.03$) in pH-NEG/SAP+ compared to pH-NEG/SAP− and HVs (Fig. 1).

Mean acid clearance time (seconds) in pH-POS [131 (82–174; 405)] was significantly higher compared to pH-NEG/SAP+ patients [37.5 (24–63; 117); $p < 0.01$],

pH-NEG/SAP− patients [45 (24–71; 155); $p < 0.01$] and HVs [32 (16–50; 85); $p < 0.01$].

Impedance data

The numbers of gastro-oesophageal reflux episodes (total, acid and weakly acidic) detected during the pH-impedance studies are indicated in Fig. 2. The median number of total and acid reflux episodes was significantly higher in pH-POS [61 (46–96; 151) and 44 (32–60; 94)] compared to pH-NEG/SAP+ [46.5 (32–65; 103) and 25 (11.5–34; 56); $p < 0.01$], pH-NEG/SAP− [33 (21.5–48; 57) and 17 (9–23; 39); $p < 0.01$] and HVs [32 (19–43; 54) and 17 (8–31; 45); $p < 0.01$]. This was also true when pH-NEG/SAP+ patients were compared to pH-NEG/SAP− and HVs ($p < 0.01$). Patients pH-POS, pH-NEG/SAP− and HVs had a similar median number of weakly acidic reflux episodes [22.5 (15–39; 86) vs. 22 (15–28.7; 48.3) vs. 18 (14–26; 45); $p = NS$]. Interestingly, pH-NEG/SAP+ patients had a significantly increased number of weakly acidic reflux events 32.5 (18.5–43.5; 82) compared to the other subgroups ($p < 0.01$).

The proportion of total reflux episodes that were acid and weakly acidic varied among the subgroups in that patients pH-POS had more acid reflux events (61 and 39%), patients pH-NEG/SAP+ had more weakly acidic reflux episodes (41 and 59%), while patients pH-NEG/SAP− (52 and 48%) and HVs (51 and 49%) had similar proportion of acid and weakly acidic refluxes. In comparison with our normal data, none of the controls, 64% of patients pH-POS, 41% of patients pH-NEG/SAP+ and

10% of patients pH-NEG/SAP− had an abnormally high total number of reflux episodes.

Median bolus clearance time (seconds) of patients pH-POS [16 (12–21; 36)] was significantly different compared to pH-NEG/SAP+ [12 (10–15; 22); $p < 0.01$], pH-NEG/SAP− [13 (10–17.7; 30); $p < 0.01$] and HVs [12 (8–16; 21); $p < 0.01$]. Conversely, no difference was found between pH-NEG/SAP+ patients, pH-NEG/SAP− patients and HVs ($p = NS$).
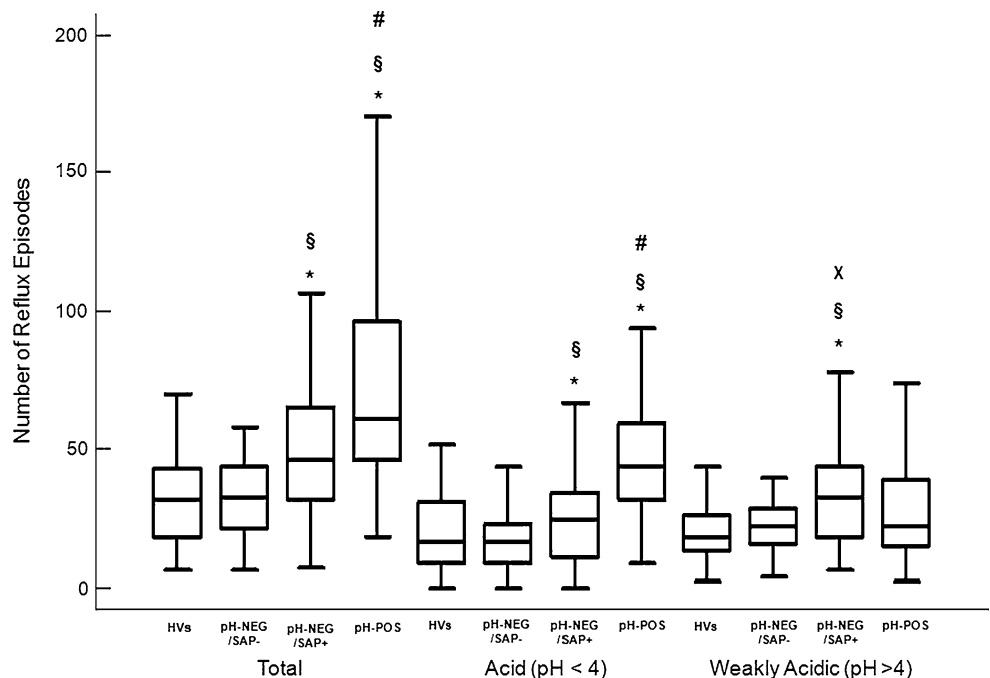
As shown in Fig. 3, the percentage of reflux episodes reaching the proximal oesophagus in patients pH-POS (50%) and pH-NEG/SAP+ (47%) was greater than in pH-NEG/SAP− (33%; $p < 0.01$) and HVs (33%; $p < 0.01$). According to our normal data, none of controls, 85% of patients pH-POS, 78% of patients pH-NEG/SAP+ and 25% of patients pH-NEG/SAP− had an abnormally high proximal number of reflux episodes.

By pooling together the abnormal total number of reflux events and the percentage of proximal reflux episodes, 92% of patients pH-POS, 86% of patients pH-NEG/SAP+ and 25% of patients pH-NEG/SAP− had an abnormal impedance testing (Fig. 4).

Correlation between impedance-pH features and symptom-reflux association in a subgroup of patients without symptoms during the testing day

Out of the 40 endoscopy-negative patients who had a normal AET and did not experience symptoms during the first test, 22 agreed (nine male, 13 female, median age 47 years, range 32–55) to undergo a second MII-pH


Fig. 2 Number of total, acid and weakly acidic reflux episodes in HVs ($n = 48$) and in pH-POS patients ($n = 130$), pH-NEG/SAP+ patients ($n = 120$) and pH-NEG/SAP− patients ($n = 79$). *$p < 0.05$ versus HVs; §$p < 0.05$ versus pH-NEG/SAP− patients; χ$p < 0.05$ versus pH-POS/SAP− patients

Fig. 3 Percentage of reflux episodes reaching the proximal measuring site (15 cm above the LOS) in HVs ($n = 48$) and in pH-POS patients ($n = 130$), pH-NEG/SAP+ patients ($n = 120$) and pH-NEG/SAP− patients ($n = 79$). *$p < 0.05$ versus HVs; §$p < 0.05$ versus pH-NEG/SAP− patients; #$p < 0.05$ versus pH-NEG/SAP+ patients
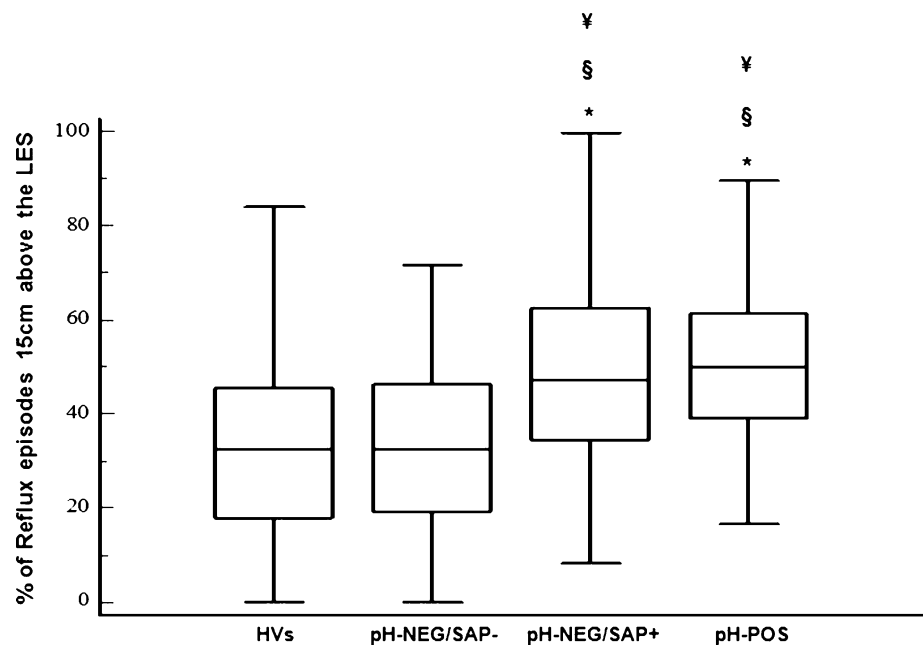
Fig. 4 Percentages of patients in the various endoscopy-negative subgroups with an abnormal impedance-pH testing by pooling together the abnormal number (>52) of reflux events and the percentage of proximal reflux episodes (>33%)
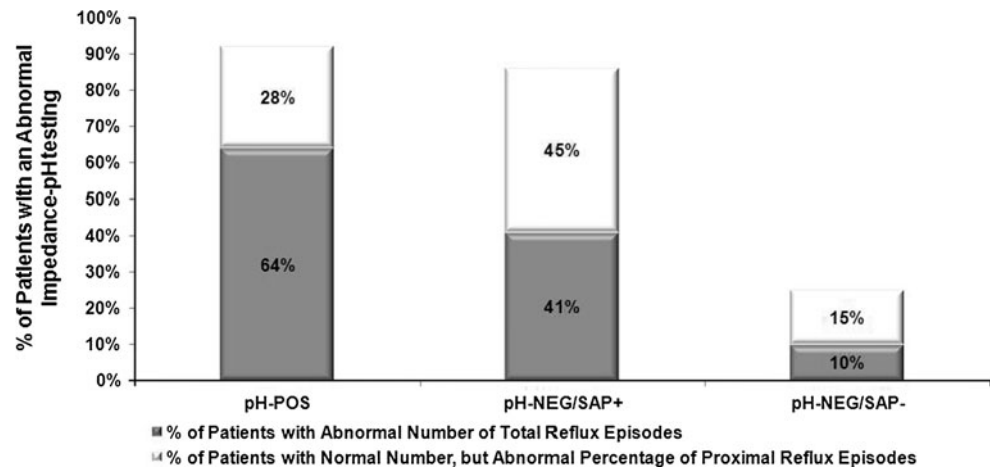
examination 4 weeks later. During this repeated test, 20 reported heartburn and were included in the analysis. Data are displayed in Table 2.

Based on the main impedance-pH features—AET, number and type of reflux episodes and proximal migration of refluxate—observed during the first MII-pH studies and in comparison with normal values from our 48 HVs, patients were stratified as follows: 10 with normal AET, normal number of reflux episodes and normal percentage of proximal reflux episodes were considered to have FH, while 10 with normal AET and abnormal number of reflux episodes and/or abnormal percentage of proximal reflux events were considered to have HO. After the second MII-pH study with the added value of SAP analysis, patients were stratified as follows: two with abnormal AET were classified as pH-POS NERD, 10 as HO and eight as FH. The rate of concordance between the two tests was 70% in cases with FH and 80% in cases with HO.

Correlation between impedance-pH features and symptom-reflux association for the diagnosis of HO and FH in the overall population with normal acid exposure

By pooling together all endoscopy-negative patients with normal AET and taking into consideration impedance-pH results combined with SAP, we found that the diagnostic accuracy in identifying HO or FH patients on the exclusive basis of impedance-pH features (i.e., HO in case of normal AET and higher number of reflux episodes and/or increased proximal migration of the refluxate and FH in case of normal AET, normal number of reflux episodes and normal proximal migration of the refluxate) was 73.71% [95% confidence interval (CI) 66–80], the sensitivity 89.3% (95% CI 84–95), the specificity 85% (95% CI 76–93), the positive predictive value 90% (95% CI 85–95), the negative predictive value 84% (95% CI 75–92).

Table 2 Stratification of endoscopy-negative patients based on impedance-pH results only on the first study and based on impedance-pH results with SAP analysis on the second analysis

Patients	Classification based on impedance-pH results only after the first MII-pH study	Classification based on impedance-pH results with SAP after the second MII-pH study
1	FH	NERD pH-POS
2	HO	HO
3	FH	HO
4	HO	HO
5	FH	FH
6	HO	HO
7	HO	HO
8	FH	HO
9	HO	FH
10	FH	FH
11	HO	HO
12	FH	FH
13	HO	HO
14	HO	NERD pH-POS
15	HO	HO
16	FH	FH
17	HO	HO
18	FH	FH
19	FH	FH
20	FH	FH
	NERD pH-POS = patient with abnormal AET	NERD pH-POS = patient with abnormal AET
	HO = hypersensitive oesophagus; patient with normal AET and higher number of reflux episodes and/or increased proximal migration of the refluxate	HO = hypersensitive oesophagus; patient with normal AET and positive SAP
	FH = functional heartburn; patient with normal AET and normal number of reflux episodes and normal proximal migration of the refluxate	FH = functional heartburn; patients with normal AET and negative SAP

Discussion

The current analysis of 24-h MII-pH monitoring data in a large group of unselected endoscopy-negative patients off-PPI medication, allowed us to observe that there is a subgroup of pH-POS NERD patients who show higher AET, higher median number of total and acid refluxes and more prolonged acid and volume clearance times compared to patients with normal AET combined with either positive or negative SAP and to HVs. Patients with normal AET and positive SAP, representing the NERD subgroup with HO, show a small but significant increase in terms of AET and

number of reflux episodes compared to FH patients and HVs, indicating that in these patients even a "physiological" degree of gastro-oesophageal reflux is able to provoke typical reflux symptoms. Finally, it is relevant to note that no differences among all the features analyzed using MII-pH testing are found between FH patients and HVs and this explains a lack of substantial symptom response to PPI therapy.

Our study shows that pH-POS NERD patients had a significantly higher AET compared to NERD with HO, FH patients and HVs, both in upright and supine position. This finding could be related to the increased prevalence of hiatal hernia in our pH-POS NERD patients, as this factor has been shown to play a major role in promoting acid refluxes [16–18]. Also the higher mean BMI value of our pH-POS NERD patients compared to those observed in NERD with HO, FH patients and HVs could be an additional factor in explaining this difference, since it is well known that being overweight or obesity increase the propensity to have reflux and consequently the tendency to higher AET [19]. On the other hand, we also found that NERD patients with abnormal AET had more prolonged acid and volume clearances than patients with HO/FH and HVs, supporting the concept that these patients have a more severe impairment of oesophageal function as suggested by previous investigations [20, 21].

We found that the number of total and acid reflux episodes was higher in pH-POS NERD patients compared to FH patients and HVs. No differences, on the contrary, were detected regarding the prevalence of weakly acidic reflux events among pH-POS NERD patients, FH patients and HVs. More importantly, patients with HO had a similar number of total reflux episodes compared to pH-POS NERD patients, but the chemical composition of refluxate differed for a greater number of weakly acidic reflux events compared to the former subgroup as well as patients with FH and HVs. This information was also confirmed by analyzing the proportions of total reflux episodes that were acidic and weakly acidic among our subgroups. Indeed, while in pH-POS NERD patients reflux events were more frequently acid, in FH and HVs reflux episodes were more equally distributed, while in patients with HO reflux events were more frequently weakly acidic. This finding is in part a logical consequence of the fact that we initially divided patients on the basis of acid exposure, but, on the other hand, the presence of an abnormal total number of reflux episodes due to the increased number of weakly acidic refluxes in HO compared to FH and HVs leads to hypothesize that the former patients have an underlying reflux disease determining their symptoms, independently from SAP. It is possible that in patients with HO, despite the presence of a normal AET, the increased number of weakly acidic reflux episodes may cause a microscopic

damage of the oesophageal mucosa—i.e., dilation of intercellular spaces and/or immune activation [22–27]—which can sensitize the oesophagus and then determine an increased perception of reflux events, as shown by the high rate of symptoms related to weakly acidic reflux found in these subjects [28]. Moreover, it is interesting to note that more than half (59%) of patients with HO had a number of reflux episodes similar to those observed in patients with FH and HVs and we can suppose that these are the true patients in whom a mechanism of hypersensitivity to "physiologic" stimuli—"sensitive oesophagus"—might occur [29–32].

It must be recognized that despite the important differences in terms of median and percentiles values found among our sub-groups of patients considering AET and the number of reflux events, there is a substantial overlap among the various subgroups analyzed and then this limits the possibility to separate completely them by means of MII-pH parameters. Accordingly, from a clinical point of view, we would like to stress that subclassifying NERD patients on the basis of reflux patterns should be considered only in those few patients who do not have symptoms during the testing day and that the information collected after the first test should be used just to "suspect" GERD and not to "diagnose" GERD.

The percentage of reflux episodes reaching the proximal oesophagus was higher in pH-POS NERD and HO patients compared to FH and HVs. These data confirm the relevance of proximal migration of reflux episodes in eliciting reflux symptoms, as previously reported by Bredenoord et al. [33] and Emerenziani et al. [10]. Therefore, in all NERD patients and in particular in those with normal AET, the percentage of proximal reflux episodes should be regarded as one of the main impedance features, if we suspect a positive relationship between symptoms and reflux events.

As to the FH subgroup, the analysis of their reflux patterns revealed that there is no difference at all with the group of HVs. Moreover, if we consider separately patients of this subgroup in terms of number of reflux episodes and percentage of reflux events reaching the proximal oesophagus, we found that only five (8%) patients had an abnormal total number of reflux episodes and 20 (25%) had an abnormal number of proximal reflux episodes. Accordingly, if we consider MII-pH as the most sensitive tool for measuring reflux [9], we have several reasons to exclude these patients from the GERD realm. In fact, the large overlap in terms of reflux characteristics with HVs sustains that factors other than gastro-oesophageal reflux are responsible for typical reflux symptoms in these subjects and that they should be included in the overall population affected by a functional disorder, in agreement with Rome III criteria [5]. Moreover, the overall normality of

impedance-pH parameters observed in these patients may explain why they did not respond, or at least in part, to aggressive acid suppression therapy. So, we can hypothesize that finding normal features with MII-pH testing may suggest the best therapeutic approach for these patients, avoiding long course of anti-secretive therapies. Further studies aimed at evaluating if impedance-pH parameters as predictors of PPI response exist will elucidate this issue.

The above differences between NERD patients and FH can be of diagnostic help in assessing those examinations in which a variable number of patients (5–30%) may not complain of typical reflux symptoms [4, 9, 10, 34–36] and, in few cases, may also forget to press the event marker button on the MII-pH data logger whenever they experience a symptom suggestive of reflux during the testing day. In these cases the examination should be repeated, particularly if the analysis of the tracings reveals a normal oesophageal AET. This is complicated by the fact that the catheter based system—pH alone or pH combined with impedance—presents some drawbacks, mainly related to its tolerability. Last but not least, the cost of these examinations make worse double testing. Consequently, the possibility of identifying a typical reflux pattern for each subgroup of the heterogeneous endoscopy-negative population appears relevant to allow us stratifying them without repeating the test in the above circumstances and to early select the best therapeutic approach for them. Therefore, the significant impedance-pH differences observed among the various subgroups of endoscopy-negative patients of our study, mainly in terms of AET, number and proximal migration of reflux episodes, that represent the main features involved in the perception of reflux episodes [33], may be useful to identify them independently of SAP analysis, at least in the majority of cases.

Indeed, we observed that the diagnosis of FH and HO done after the first asymptomatic impedance-pH study was confirmed by the second symptomatic test in 70 and 80% of cases, respectively. A negative impedance-pH study seems to be slightly less predictive of FH, probably because of the day-to-day variability that can produce false-negative results. However, an increased number of both acid and weakly acidic refluxes and/or a higher proximal migration of refluxate seem to be greatly associated with a diagnosis of NERD with HO. Indeed, as shown comparing reflux patterns among our subgroups, we observed that by pooling together the abnormal total number of reflux events and the higher percentage of proximal reflux episodes is it possible to find 86% of patients with HO compared to 25% of those with FH. So, the above two parameters must be taken into great account to differentiate with a good degree of accuracy HO from FH patients on the sole basis of reflux patterns detected by MII-pH testing. These results have been reinforced by the

analysis of all endoscopy-negative patients with normal AET, since we found that, compared to impedance-pH results combined with SAP, the diagnostic accuracy in identifying HO or FH patients on the only basis of impedance-pH features was as high as 73.7% and the sensitivity (89.3%), the specificity (85%), the positive predictive value (90%) and the negative predictive value (84%) were also good.

However, it must be stressed that the overlap of reflux patterns between our subgroups and the possible variability of one of the parameters taken into account to distinguish patients with FH from those with HO may contribute to render difficult the diagnosis and therefore impedance-pH testing must be anyhow repeated at least in the most doubtful cases in order to exploit the important aspect of SAP.

Finally, some clinical and demographic features were different in our subgroups. We found that NERD pH-POS patients were more frequently male and had a higher mean BMI, in agreement with those studies considering these features among the main risk factors for the development of GERD and, particularly, abnormal pH monitoring [4, 38–40]. In particular, analyzing a very large population of patients with both erosive oesophagitis and NERD, we have recently found that overweight represents an important risk factor for erosive oesophagitis [odds ratio (OR) 1.4; 95% CI 1.2–1.6] and pH-POS NERD (OR 1.35; 95% CI 1.2–1.5) and not for HO and FH [41]. Moreover, we observed a higher prevalence of female gender in the FH subgroup, as it has been reported in the functional dyspepsia population and generally in patients with GI functional disorders [4, 42, 43]. No differences based on age, tobacco use, alcohol and coffee consumption could be demonstrated in our population. This is in agreement with other studies [40, 43] showing that these factors are not peculiar of anyone of our subgroups.

We are conscious that one limitation of this study is the relatively small sample of patients investigated twice; however, our findings underline that, mainly in patients with normal AET, a study with none symptoms reported during the monitoring day does not mean immediately that it is to be repeated. On the contrary, the results we obtained in patients studied twice suggest an accurate diagnosis in most cases and this can be of help for an initial therapeutic approach in these patients—antireflux therapy for suspected HO disease, pain-modulator drugs for suspected FH disorder—especially in the frequent circumstance that patients reject to undergo an additional invasive test. The use of SAP analysis may be also regarded as a potential limitation of this study, since it has been shown that it is not a perfect tool. Indeed, while it may decrease the number of false negatives, its use may also result in falsely classifying functional patients as GERD [37]. However, we opted to use it because SAP has been reported to be more accurate than Symptom Index in evaluating the temporal relationship between symptoms and reflux episodes [15].

In conclusion, important different reflux patterns may permit to classify endoscopy-negative patients in various subgroups with good reliability. In particular, patients with HO are characterized by an increased number of acid and especially weakly acidic reflux events, despite a normal AET, and by a very high percentage of proximal reflux episodes. So, these two features should be regarded as peculiar of HO. The above differences may have the practical advantage of detecting the subgroup of NERD or FH to which patients without symptoms during the impedance-pH testing belong, thus avoiding a second uncomfortable examination.

Conflict of interest The authors declared no conflict of interest.

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