Towards better understanding of symptoms associated with disordered esophageal function
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DETERMINANTS OF REFLUX-INDUCED CHRONIC COUGH

Thomas V.K. Herregods, Ans Pauwels, Jafar Jafari, Daniel Sifrim, Albert J. Bredenoord, Jan Tack, André J.P.M. Smout

ABSTRACT

Background
Gastro-oesophageal reflux is considered to be an important contributing factor in chronic unexplained cough. It remains unclear why some reflux episodes in the same patient causes cough while others do not. To understand more about the mechanism by which reflux induces cough, we aimed to identify factors which are important in triggering cough.

Design
In this multicentre study, 49 patients with reflux-associated chronic cough were analysed using 24-hour pH-impedance-pressure monitoring. The characteristics of reflux episodes that were followed by cough were compared with reflux episodes not associated with cough.

Results
The majority (72.4%) of the reflux episodes were acidic (pH<4). Compared with reflux episodes that were not followed by cough, reflux episodes that were followed by a cough burst were associated with a higher proximal extent (p=0.0001), a higher volume clearance time (p=0.002), and a higher acid burden in the preceding 15-minute window (p=0.019) and higher reflux burden in the preceding 30-minute window (p=0.044). No significant difference was found between the two groups when looking at the nadir pH, the pH drop, the acid clearance time, or the percentage of reflux episodes which were acidic.

Conclusions
The presence of a larger volume of refluxate and oesophageal exposure to reflux for a longer period of time seems to play an important role in inducing cough, while the acidity of the refluxate seems to be less relevant. This helps explain the observation that most patients with chronic cough tend not to benefit from acid inhibitory treatment.
INTRODUCTION

Chronic cough is defined as cough lasting for more than 8 weeks. It is a common problem, estimated to affect 9-33% of European and US populations, which significantly impairs health-related quality of life and is associated with a substantial socio-economic burden. When pulmonary and ear, nose and throat causes such as asthma and post-nasal drip have been ruled out, gastro-oesophageal reflux (GOR) should be considered as a causal factor.

The relationship between chronic cough and gastro-oesophageal reflux is complex. A diagnosis of gastro-oesophageal reflux disease (GORD) based on pathological acid exposure or the presence of oesophagitis or Barrett’s metaplasia does not establish a causal relationship between reflux and chronic cough. Furthermore, many patients with chronic cough do not present with the typical GORD symptoms of heartburn and regurgitation. Assessment of the temporal relationship between reflux episodes and cough bursts by means of prolonged reflux monitoring (pH only or pH-impedance combined) helps to establish a diagnosis of reflux-induced cough in patients with unexplained chronic cough. The most frequently used instrument in this assessment is the symptom association probability (SAP). It has been shown that, for an optimal assessment, the cough bursts must be recorded objectively, using either an acoustic or a manometric technique.

Two alternative hypotheses have been suggested for the pathophysiological mechanism underlying reflux-induced cough. The first is that reflux-induced cough results from stimulation of a vagal oesophagobronchial reflex in which reflux, even when confined to the distal oesophagus, triggers a cough reflex. The second hypothesis suggests that (micro)aspiration of gastric content stimulates the cough reflex by irritating the respiratory tract directly. Previous studies have shown that pepsin concentrations in the larger airways in patients with chronic cough are similar to those in healthy volunteers. Moreover, acid infused into the distal oesophagus of chronic cough patients increases the frequency of coughing, and in patients with a positive SAP for reflux-induced cough the sensitivity of the cough reflex elicited by citric acid is increased.

Several questions concerning the generation of reflux-induced cough have remained unanswered. For instance, it is unclear why some GORD patients have chronic cough while others with a similar reflux profile do not. In patients with GORD, it has been shown that reflux episodes are more likely to cause heartburn or regurgitation complaints when they have a larger pH drop, a lower nadir pH, a higher proximal extent, and a longer volume and acid clearance time. While these studies have increased our understanding of the perception of reflux, it remains unclear why some reflux episodes in the same patient are associated with cough while others are not. Therefore, this study was undertaken to increase our understanding of the mechanisms by which reflux induces cough.
METHODS

Subjects
In 260 patients with chronic unexplained cough simultaneous 24-hour ambulatory oesophageal pH-impedance-pressure monitoring was performed between 02-2010 and 08-2015. From these, a subgroup of 49 patients was selected in whom the analysis of the data provided evidence for reflux-induced cough, as evidenced by a positive SAP for reflux-cough sequences. Patients were included in three different centres; the Academic Medical Centre in the Netherlands, the Barts and the London NHS Trust in the United Kingdom and the University Hospital Leuven in Belgium. Patients were excluded when an alternative diagnosis such as asthma, postnasal drip or the use of ACE inhibitors could explain the symptoms. At least 7 days prior to the measurements proton pump inhibitors (PPIs) were stopped. Other drugs affecting gastro-intestinal motility or secretion were not allowed to be taken for at least 3 days prior to the measurements. Patients were excluded if they had undergone any upper gastrointestinal surgery. The presence of typical reflux symptoms (heartburn and/or regurgitation) was assessed by reviewing the referral letter and clinician notes. To be included patients had to have at least 4 cough bursts throughout the 24-hour measurement and the measurement needed to have lasted for at least 16 hours. The study proposal was submitted to the local institutional review board of the Academic Medical Centre in Amsterdam, the Netherlands. Formal evaluation was waived according to Dutch law (26-02-2015 reference number W15_063 # 15.0075).

Ambulatory 24-hour pressure and pH-impedance monitoring
All subjects underwent ambulatory 24-hour oesophageal pH-impedance-pressure monitoring off acid suppressive therapy. Intra-oesophageal pressure was recorded using an 8 French solid-state manometric catheter with 3-4 pressure sensors which were separated by 5-cm intervals (Unisensor, Attikon, Switzerland in the AMC and Leuven, and Sandhill Scientific, Inc. in the centre in London). The most distal sensor was placed in the stomach. The 24-hour pH-impedance measurement was carried out using a combined pH-impedance catheter assembly (Unisensor in the AMC and Sandhill Scientific, Inc. in the centres in London and Leuven). The catheter contained six impedance recording segments which were located at 2-4, 4-6, 6-8, 8-10, 14-16 and 16-18 cm above the upper border of the lower oesophageal sphincter (LOS), and one ion-sensitive field-effect transistor (ISFET) pH electrode which was placed 5 cm above the upper border of the manometrically localized LOS. Both aforementioned catheters were introduced via the same nostril and were taped to the face. The impedance, pH, and pressure signals were stored on a digital datalogger (Ohmega, MMS, Enschede, the Netherlands, in the AMC, and Sleuth, Sandhill Scientific, Inc. Highlands Ranch, CO in London and Leuven), using a sampling frequency of 8 Hz for the pressure signals and 50 Hz for the pH-impedance signals.
Patients were instructed to press the event marker button on the pH data logger whenever they were coughing or when they experienced another symptom. In addition, they had to note down the nature of the symptom event and its time of onset in a specially designed diary. Patients were instructed to mark the times of meals and beverages in the diary and were told to restrict their intake to three meals and four beverages at standardized times throughout the 24 hours. They were also encouraged to maintain normal daily activities throughout the measurement and were told to mark the period spent in the supine position.

**Data analysis**

The pH-impedance and manometric recordings were uploaded to a computer and were then manually analysed (MMS, Enschede, the Netherlands in the AMC, and Bioview, Sandhill Scientific Inc., Colorado, USA in the centre in London and Leuven).

**Cough detection**

The manometric recordings were independently analysed for cough bursts. A cough was defined as a short-duration, rapid, simultaneous pressure peak with a time to peak <1 second\(^1\). It also required the same pressure configuration at all intra-oesophageal recording sites. As in previous studies\(^{10,11}\), only “cough bursts” defined as two or more rapid simultaneous pressure peaks within 3 seconds were analysed and single cough events were ignored\(^9\). In order for cough bursts to be considered as separate events, they were required to be separated by 30 seconds. Objectively detected cough bursts were also compared to cough events marked by the patients in the data logger and diary.

**Reflux detection**

The pH-impedance tracings were analysed independently from the manometric tracings. Gastro-oesophageal reflux (GOR) was defined as an orally progressing, sequential, drop in impedance to less than 50% of the baseline values and starting at the most distal impedance segment (2-4 cm above the LOS) and propagating retrogradely to at least the next impedance segment (4-6 cm above the LOS). GOR was defined as acid if the pH fell below 4 for at least 4 seconds. If the pH was already below 4 at onset of the reflux episode, then a further drop in pH by at least 1 unit for at least 4 seconds was required\(^2\). GOR was defined as weakly acidic if the drop in impedance was not accompanied by a drop in pH below 4.

We did not include pure gas reflux episodes without a liquid component (belches) in the analysis. Mixed reflux was defined as gas reflux (rapid (> 3000 Ω/s) retrograde moving increase in impedance in at least two consecutive impedance segments) occurring during or immediately before liquid reflux. For each reflux episode we noted the proximal extent, the time needed to reach this proximal sensor, the ascending velocity, the nadir pH and the change in pH. The volume clearance time was defined
as the time in seconds from the 50% drop in impedance of baseline in the most
distal segment until this impedance recovered to above this point again\textsuperscript{21}. The acid
clearance time was defined as the time in seconds from the moment the oesophageal
pH dropped below 4 until it recovered to a value of 4 or until a new reflux episode
started. In case the pH was already below 4 at the start of the reflux episode, a further
drop of 1 pH unit indicated the start. For each patient the total acid exposure time
and the total number of reflux events (both acid and weakly acidic) were calculated.
The total acid exposure time was defined as the percentage of time that the pH was
below 4 throughout the entire measurement. This was considered pathological if it
was greater than 6\% of the total recording time\textsuperscript{22}. Oesophagitis was classified using
the Los Angeles Classification\textsuperscript{23}.

The acid burden was measured as the total time with pH below 4 measured in
the 15-, 30-, 45- and 60-minute time segment preceding the onset of each reflux
episode\textsuperscript{17}. We defined the reflux burden as the total reflux time (time from the 50% drop in impedance of baseline in the most distal segment until the impedance
recovered to above that point) measured in the 15-, 30-, 45- and 60-minute time
segment preceding the onset of each reflux episode. The three meals were excluded
for all analyses, including the acid and reflux burden measurements. If a meal fell
within the time period used for the measurement of the acid burden or reflux burden
time, then that period was considered to be not applicable and was not used in
the calculation. As a result, for the 60-minute time window more reflux episodes
were not applicable than for the other time windows. Due to the short duration of
beverage consumption, these moments were ignored for the acid burden and reflux
burden calculation.

Association between reflux and cough
A reflux episode was considered associated with a cough burst if the cough burst
followed within two minutes after onset of the reflux episode (reflux-cough episode).
The association between reflux and cough bursts was assessed using the SAP\textsuperscript{12}
and a 2-minute time window\textsuperscript{24}. We considered a SAP greater than 95\% to be
statistically significant. Cough bursts which occurred outside of the 2-minute time
window following the onset of a reflux episode were considered unrelated to that
reflux episode.

**Statistical analysis**
Data are presented as median (interquartile range (IQR)) unless stated otherwise.
The Fisher exact test was used to calculate the SAP. Comparison of associated and non-
associated reflux episodes within subjects was performed using the Wilcoxon signed
rank test. Relationships between parameters were expressed using the Spearman’s
Rank correlation. A p-value < 0.05 was considered statistically significant.
RESULTS

Patient population
The main patient characteristics are shown in table 1.
A total of 260 patients underwent 24-hour impedance-pH-manometry for their chronic unexplained cough. Fourteen patients were excluded because they had not stopped their PPIs for 7 days prior to the measurement, 29 patients because they had <4 cough bursts, and two because they had undergone upper gastrointestinal surgery. Twenty-three patients were excluded because of technical problems with the catheter or because the measurement lasted less than 16 hours. Of the remaining 192 patients, 143 patients were excluded because they did not have a positive SAP for reflux-cough. As a result, analysis was completed in 49 patients. In these patients, the median recording time was 22.47 hours (IQR 21.5-23.2).

Cough
A total of 1890 cough bursts were detected manometrically. Of these, 1246 (65.9%) were noted by the patients by pressing the event marker button. Patients had a median of 31 cough bursts (18-52) measured manometrically.

Gastro-oesophageal reflux
A total of 2270 reflux episodes were detected and analysed. Of these, 1643 (72.4%) were acid and 627 were weakly acidic. A median of 47 (35-59) reflux episodes were found per patient, of which 30 (22-45) were acid reflux episodes. Most reflux episodes took place in the upright position (94.3%). A total of 1024 (45.1%) reflux episodes were pure liquid while 1246 reflux episodes were mixed liquid-gas. A median of 8 (3-15) reflux episodes reached the most proximal impedance sensor.

Fifteen patients (30.6%) had a pathological acid exposure time. Patients had a median acid exposure time of 2.5% (1.2-7.4), with 4% (1.8-10.4) in the upright position and 0.1% (0-0.6) in the recumbent position. At upper endoscopy, reflux oesophagitis

Table 1. Clinical and demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>49</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>54</td>
</tr>
<tr>
<td>Female gender</td>
<td>32 (65.3%)</td>
</tr>
<tr>
<td>Number of patients presenting symptoms</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>49 (100%)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>23 (46.9%)</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>17 (34.7%)</td>
</tr>
</tbody>
</table>
was found in 11 patients, nine of whom had Los Angeles grade A oesophagitis and 2 patients grade B. Thirty patients (61.2%) had heartburn and/or regurgitation complaints with 23 patients having heartburn and 17 patients having regurgitation.

**Cough-associated versus not associated reflux episodes**

In total, 395 reflux episodes were associated with cough bursts while 1875 reflux episodes were not. A total of 414 cough bursts followed a reflux episode within 2 minutes. In table 2 the characteristics of the reflux episodes which were associated with cough bursts are compared to those which were not associated to cough bursts.

The proximal extent of the refluxate was significantly higher (p=0.0001) in the cough-associated reflux episodes. This is shown in Figure 1. A median of 20% (10-42.8) of the reflux episodes reaching the most proximal impedance sensor was followed by a cough burst. A total of 123 reflux episodes which were followed by cough bursts reached the most proximal sensor. In addition, we found that the volume clearance time was significantly longer in the cough-associated reflux episodes compared to the non-associated episodes (p=0.002), with a median volume clearance time of 17 seconds (12-22) in the cough-associated reflux episodes compared to 14 seconds (12-17) in the episodes not associated to cough. No significant difference was found

### Table 2. Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Associated reflux episodes</th>
<th>Non-associated reflux episodes</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal extent (cm)</td>
<td>9 (8.5-16)</td>
<td>9 (7-9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Ascending velocity (cm/s)</td>
<td>3.1 (2.1-4.8)</td>
<td>2.8 (2.0-3.9)</td>
<td>0.159</td>
</tr>
<tr>
<td>Nadir pH</td>
<td>3.15 (1.97-4.12)</td>
<td>2.75 (2.20-3.77)</td>
<td>0.394</td>
</tr>
<tr>
<td>pH drop</td>
<td>3.4 (2.47-4.22)</td>
<td>3.3 (2.55-3.80)</td>
<td>0.571</td>
</tr>
<tr>
<td>Volume clearance time (s)</td>
<td>17 (12-22)</td>
<td>14 (12-17)</td>
<td>0.002</td>
</tr>
<tr>
<td>Acid clearance time (s)</td>
<td>33.5 (10-62.4)</td>
<td>27.6 (10.6-60.5)</td>
<td>0.453</td>
</tr>
<tr>
<td>Percentage acidic (%)</td>
<td>66.7 (43.7-88.7)</td>
<td>70 (50.0-86.6)</td>
<td>0.367</td>
</tr>
<tr>
<td>15-min acid burden(s)</td>
<td>2.8 (0-20.3)</td>
<td>0 (0-15.6)</td>
<td>0.019</td>
</tr>
<tr>
<td>30-min acid burden(s)</td>
<td>25.3 (0.5-92.6)</td>
<td>16.9 (1.7-122.2)</td>
<td>0.342</td>
</tr>
<tr>
<td>45-min acid burden(s)</td>
<td>53.2 (2.3-154.1)</td>
<td>32.9 (4.3-182.5)</td>
<td>0.167</td>
</tr>
<tr>
<td>60-min acid burden (s)</td>
<td>38.1 (3.5-194.5)</td>
<td>46.6 (10.2-268.0)</td>
<td>0.866</td>
</tr>
<tr>
<td>15-min volume burden (s)</td>
<td>11.0 (4.4-17.6)</td>
<td>11.5 (3.3-16.5)</td>
<td>0.116</td>
</tr>
<tr>
<td>30-min volume burden (s)</td>
<td>27.8 (15.6-46.0)</td>
<td>25 (17.0-40.9)</td>
<td>0.044</td>
</tr>
<tr>
<td>45-min volume burden (s)</td>
<td>43.9 (26.7-66.8)</td>
<td>42.9 (28.0-60.7)</td>
<td>0.486</td>
</tr>
<tr>
<td>60-min volume burden (s)</td>
<td>56.2 (26.6-87.5)</td>
<td>57.0 (35.8-85.2)</td>
<td>0.652</td>
</tr>
<tr>
<td>Percentage in upright position (%)</td>
<td>100 (88.2-100)</td>
<td>97.4 (93.0-100)</td>
<td>0.407</td>
</tr>
<tr>
<td>Percentage liquid (%)</td>
<td>42.9 (21.1-63.3)</td>
<td>39.1 (26.0-60.6)</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Reflux episodes which were associated with cough bursts are compared to reflux episodes which were not associated with cough bursts. All values are shown as median (IQR). The p-value was calculated using the Wilcoxon signed rank test.
for ascending velocity, the nadir pH, or the size of the pH drop. Moreover, there was no difference in acid clearance time when comparing both groups. The percentages of acidic reflux episodes were similar in both groups (66.7% (43.7-88.7) in the cough-associated group and 70% (50-86.6) in the non-associated group).

Reflux episodes followed by cough bursts were preceded by a significantly higher 15-minute oesophageal cumulative acid exposure time preceding the cough (acid burden) (p=0.019), but no significant difference in acid burden time was found for the 30-minute, 45-minute or 60-minute interval. Reflux episodes which were associated with cough bursts had a significantly higher 30-minute oesophageal cumulative reflux exposure time (reflux burden) (p=0.044) and a trend was found for the 15-minute reflux burden time (p=0.116). Body position and composition of the refluxate (liquid or mixed) were similar in both groups.

When looking at reflux episodes that were followed by cough bursts, statistically significant relationships were found between the proximal extent of the refluxate and the volume clearance time ($r=0.375$, $p=0.008$) and percentage of liquid reflux episodes ($r=-0.313$, $p=0.029$). As expected, a relationship was found between the volume clearance time and the acid clearance time ($r=0.319$, $p=0.025$), and a strong relationship between all reflux burden and all acid burden times (15-minute acid burden vs. 15-minute reflux burden ($r=0.721$, $p<0.0001$), 30-minute ($r=0.599$, $p<0.0001$), 45-minute ($r=0.689$, $p<0.0001$), and 60-minute ($r=0.729$, $p<0.0001$)).

Figure 1. Boxplot graph comparing the proximal extent of reflux episodes which were associated with cough bursts compared with reflux episodes which were not.
DISCUSSION

The relation between gastro-oesophageal reflux and cough is complex and despite many studies, it remains unclear how reflux induces cough. Moreover, it is unclear why some patients with gastro-oesophageal reflux also present with chronic cough while others do not, and why acid-suppressive therapy was found to be ineffective in reducing the cough frequency in many placebo-controlled trials[1,25]. This is the first study in which a wide range of reflux characteristics was evaluated in patients in whom a temporal relationship between reflux and cough events was found.

Previous studies looking at the perception of reflux episodes have found a link between perception of typical reflux symptoms and the proximal extent of the refluxate, the nadir pH, the magnitude of the pH drop, the acid clearance time and the acid burden[17,18]. It has become clear that the acidity of the refluxate is an important determinant of perception of typical reflux symptoms. Acid appears to play a less crucial role in reflux-induced chronic cough as only 30.6% of the patients had a pathological acid exposure time, and the median acid exposure time was only 2.5%. However, we did find a significantly higher oesophageal acid exposure time in the 15-minute window preceding the cough (15-minute acid burden time), which suggests that oesophageal acid exposure may sensitize the oesophagus for subsequent reflux episodes. In contrast with the results of a previous study on factors determining the perception of reflux episodes[17], we did not find a significant difference when evaluating time windows longer than 15 minutes. Moreover, reflux episodes which were followed by cough were not more frequently acidic, did not have a lower nadir pH, a larger pH drop, or a longer acid clearance time compared to reflux episodes which were unrelated to cough. This suggests that acidity of the refluxate is not a key factor in inducing cough bursts. The findings are in accordance with the results of earlier studies showing that weakly acidic reflux episodes are still able to result in cough[10,11,13]. The higher preceding acid burden time which was observed in our study, could be the result of the significantly higher volume clearance time and the higher cumulative oesophageal reflux exposure (reflux burden). We found a trend towards a higher 15-minute reflux burden and a significantly higher 30-minute reflux burden. This suggests that the time that the refluxate is present in the oesophagus is more important than the time that acid is present and therefore the quantity of the refluxate, or a non-acidic component of the refluxate, could be an important factor. These observations may help to explain the limited efficacy of acid-inhibitory medication in placebo-controlled trials[1,25], and support the notion that refluxate sensitises the oesophagus to subsequent reflux episodes. It should be borne in mind that clearance starts with a peristaltic contraction which removes most of the reflux volume. Subsequently, acid neutralization by swallowed saliva takes place[26]. It seems that the clearance of the refluxate through peristaltic contractions is the more
important step in this patient population as the smaller amount of acid remaining seems to be less relevant.

Importantly, in a per subject analysis the proximal extent of cough-associated reflux episodes was significantly higher than the proximal extent of non-associated reflux episodes. This implies that the proximal extent of the refluxate is an important determinant of reflux-induced cough. However, similar to previous studies\textsuperscript{10,13}, we found that at the group level, the number of reflux episodes reaching the most proximal sensor did not differ from healthy subjects\textsuperscript{27}, and most of the reflux episodes reaching the most proximal sensor did not trigger cough. We did find that the proximal extent correlated well with the volume clearance time, and this suggests that the volume of the refluxate is a determinant of the occurrence of cough bursts. Since volume rather than acidity seems to be important in patients with reflux-induced cough, this could explain why anti-reflux surgery seems to be more effective than medical treatment\textsuperscript{28}. However, it is important to note that differences in selection criteria might account for this and that the surgical trials lacked a sham control group\textsuperscript{29-31}. Another possibility is that some patients have a more reflux-sensitive proximal oesophagus.

Nineteen patients did not have any typical reflux symptoms (heartburn and/or regurgitation), demonstrating that typical reflux symptoms are not necessary for reflux to induce cough. Moreover, most patients did not have oesophagitis on gastroscopy and reflux episodes only reaching the distal oesophagus were still able to trigger cough. This supports the notion of a sensitized oesophagobronchial reflex in patients with reflux-induced cough as suggested by previous studies\textsuperscript{10,11,13}. It could be argued that miniscule traces of reflux could still reach the aerodigestive tract leading to micro-aspiration, yet this would not explain why most reflux episodes reaching the proximal oesophagus did not result in cough. Moreover, it has been shown that airway pepsin concentrations in the bronchoalveolar lavage fluid of chronic cough patients are not different from those in healthy volunteers\textsuperscript{14}, supporting the notion that micro-aspiration is less likely. Furthermore, Ing et al. demonstrated that the cough frequency is increased in chronic cough patients compared to controls after acid infusion into the distal oesophagus, and to a lesser degree after saline infusion\textsuperscript{16}. This could be because exposure of the oesophagus to refluxate, regardless of acidity, seems to sensitize the oesophagus for subsequent reflux episodes. Therefore, the use of medication targeting this sensitization such as amitriptyline or citalopram might have a beneficial effect in this patient population, although formal studies addressing this hypothesis are lacking.

Most coughs were not temporally associated with reflux, which supports the notion that cough could be self-perpetuating due to central processes\textsuperscript{33}, or that multiple mechanisms may operate in an individual patient. The importance of central mechanisms is supported by studies which have shown an improvement in cough frequency and severity when treated with gabapentin, a centrally active drug\textsuperscript{32,33}. 
Moreover, Javorkova et al. showed that patients with GOR and cough tended to have lower cough thresholds for capsaicin after acid and even after saline infusion compared to patients with GOR but without cough. This suggests that reflux can sensitize a cough reflex, resulting in other stimuli, e.g. environmental stimuli such as temperature changes or perfume, being able to initiate and exacerbate cough. Another possibility, suggested in an article by Kahrilas et al., is that reflux is just another one of the aforementioned stimuli and that the sensitization is the result of another mechanism.

In this study we did not find a significant difference in ascending velocity between reflux episodes that were followed by cough and those that were not. Moreover, body position or composition of the refluxate (liquid or mixed) did not seem to influence the chance for reflux to associate with cough. As can be expected, mixed reflux correlated with the proximal extent, and the objective measurement of cough bursts is crucial as only 65.9% of the cough bursts were noted by the patients themselves. Analysis based on the subjective measurement alone would be less accurate as many cough bursts would be missed, and the exact onset would be unknown.

It is important to realize that considerable overlap was found in the properties of reflux episodes which induced cough compared to those which did not. As a result, it is not possible to establish thresholds which, if reached, would consistently provoke cough. We opted for strict criteria as we wished to study a group of patients who had a high chance of having reflux as the predominant cause of their cough. In our study, only 25.5% of the patients considered for inclusion had a positive SAP for reflux-induced cough. This is less than in previous studies, in which a positive SAP was found in 30-48% of evaluated patients. The highest percentage of patients with a positive SAP was found in the study by Smith et al. who used acoustic monitoring to detect coughs and included single-cough events. In studies using manometry only cough bursts are evaluated because it is felt that single simultaneous pressure peaks can be artefactual and patient complaints tend to be related to cough bursts rather than to single events. This is supported by a study which found that 87% of episodes marked by the patients corresponded with manometrically detected cough bursts. Furthermore, in comparison to the acoustic study in which a 2-second quiescent period was used, we considered cough bursts as separate events only when they were separated by 30 seconds.

In conclusion, the results of our study indicate that the volume clearance time, the reflux burden and the proximal extent are important factors in causing a reflux episode to induce a cough burst. The presence of a larger volume of refluxate for a longer period of time seems to play a major role in inducing a cough burst, while the drop in pH, nadir pH or acid clearance time does not seem to be important. This helps explain the observation that patients with chronic cough tend not to benefit from acid-suppressive treatment. Furthermore, this study has provided further evidence that micro-aspiration seems to be a less likely cause of reflux-induced cough. Instead central sensitization or an oesophagobronchial reflex is a more likely mechanism.
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