Increased Sensitivity of Cough Reflex is Not the Mechanism of Cough Attributed to Laryngopharyngeal Reflux

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Summary: Objectives. In laryngopharyngeal reflux (LPR) patients acid reaches laryngopharyngeal area and stimulates/sensitizes respiratory nerve terminals mediating cough. We addressed several hypothesis: if stimulation of respiratory nerves is responsible for coughing then acidic LPR should correlate with coughing and proton pump inhibitor (PPI) treatment should reduce both LPR and coughing. If sensitization of respiratory nerves is responsible for coughing then cough sensitivity should correlate with coughing and PPI should reduce both coughing and cough sensitivity.

Study design/Methods. In this prospective single center study, patients with positive reflux symptom index (RSI > 13) and/or reflux finding score (RFS > 7) and ≥1 LPR episode/24 hours were enrolled. We evaluated LPR by dual channel 24-hour pH/impedance. We determined number of LPR events with pH drop at levels 6.0, 5.5, 5.0, 4.5, and 4.0. Cough reflex sensitivity was determined as lowest capsaicin concentration causing at least 2/5 coughs (C2/C5) by single breath capsaicin inhalation challenge. For statistical analysis C2/C5 values were -log transformed. Troublesome coughing was evaluated on the scale 0-5.

Results. We enrolled 27 LPR patients. The number of LPR events with pH 6.0, 5.5, 5.0, 4.5, and 4.0 was 14[8-23],4[2-6],1[1-3],1[0-2] and 0[0-1], respectively. There was no correlation between number of LPR episodes at any pH level and coughing (Pearson range -0.34 to 0.21, P = NS). There was no correlation between cough reflex sensitivity C2/C5 and coughing (R = -0.29 to 0.34, P = NS). Of patients that completed PPI treatment, 11 had RSI normalized (18.36 ± 2.75 vs. 7 ± 1.35, $P < 0.01$). There was no change in cough reflex sensitivity in PPI-responders. C2 threshold was 1.41 ± 0.19 vs. 1.2 ± 0.19 ($P = 0.11$) before and after PPI.

Conclusions. No correlation between cough sensitivity and coughing and no change in cough sensitivity despite improvement of coughing by PPI argue that an increased cough reflex sensitivity is not mechanism of cough in LPR. We identified no simple relationship between LPR and coughing suggesting that this relationship is more complex.

Key Words: Acid−Laryngopharyngeal reflux−Cough−Cough reflex sensitivity−Gastroesophageal reflux.

INTRODUCTION

Laryngopharyngeal reflux (LPR) is nowadays regarded as entity that is distinct from gastroesophageal reflux disease (GERD) and is thought to be responsible for so called extra-esophageal symptoms, mainly cough, throat clearing, and globus sensation. GERD and LPR have not only different clinical manifestations, but also risk factors and pathophysiology.

Cough is one of the most troublesome symptoms of LPR and significantly impairs the quality of life. Substantial proportion of LPR patients suffer from chronic cough. Cough, in the context of LPR, is a symptom that is rather difficult to study. There is lack of consensus for the diagnostic gold standard of LPR and consequently, the results of studies are heterogeneous that precludes the straightforward conclusion on the pathophysiological basis of cough in LPR patients. Moreover, LPR symptoms commonly overlap with other medical conditions and coinciding diseases (eg, postnasal drip, chronic tonsillitis etc.) what makes the designing of the pathophysiological study and recruiting the study population difficult.

Various mechanisms that might lead to reflux-associated cough have been proposed. Of these, the direct activation of sensory nerves by acidic refluxate in the upper airways/larynx has been widely accepted.[1] Chronic inflammation of the larynx and upper airways commonly accompanies LPR[2] and induces the production of inflammatory mediators contributing to triggering cough. Last but not least, the activation of extrapulmonary sensory fibers contributes to cough produced by the phenomenon of cough hypersensitivity, that is accompanied by significant plastic changes of the afferent nerve endings.[3] All of the abovementioned ways might be relevant in LPR patients. To the best of our knowledge, there was no attempt for the pathophysiological investigation of cough in patients with LPR.

We have largely overcome the hurdles that preclude the investigation of the pathogenesis of cough in the present study. We recruited a population of precisely selected patients with high probability of LPR by setting strict symptom based, laryngoscopic and 24-hour pH/impedance inclusion criteria.

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We addressed two hypotheses to elucidate the relationship between LPR and cough. Firstly, we evaluated whether direct stimulation of respiratory nerves by acidic LPR is responsible for coughing. By stimulation we mean the direct initiation of cough by the activation of laryngeal sensory nerves. We therefore analyzed the correlation between the amount of acidic LPR in the hypopharynx and coughing and the effect of PPI treatment on the both hypopharyngeal LPR and coughing. Secondly, we evaluated whether sensitization of respiratory nerves (increased cough reflex sensitivity) is responsible for coughing. Sensitization of cough refers to a condition in which the cough reflex is more readily induced. In the context of this study, it is demonstrated as a decreased intensity of the stimulus required to trigger cough and is evaluated by measuring the cough threshold to inhaled irritant (aerosolized tussigen, capsaicin) during the cough challenge.[4] Taken together, we analyzed the relationship between the cough reflex sensitivity as determined by capsaicin cough challenge and coughing and the effect of PPI treatment on both sensitivity of cough reflex and coughing.

METHODS

The protocol of the study was approved by the Ethics Committee of Jessenius Faculty of Medicine, Comenius University (approval number EK 1485/2014). The evaluation was performed on outpatient basis. Consecutive patients referred for suspected LPR to the Clinic of Internal Medicine—Gastroenterology were prospectively screened for eligibility. A careful interview was conducted to assess the symptoms suggestive of LPR. Inclusion criteria were laryngopharyngeal symptoms such as cough, hoarseness, globus and throat clearing for >6 months. Exclusion criteria were age below 18 years old, infection of the upper or lower airways in the previous month, smoking, alcohol consumption >40g/d, history of thoracic or abdominal surgery, pregnancy or breast feeding, neurological disorders, active malignancy, inflammatory bowel disease and presence of gastric inlet patch in the esophagus on the upper endoscopy. Written informed consent was obtained from each eligible subject who agreed to be enrolled into the study.

Subjects were instructed to withhold PPI therapy for 30 days. After this period, the subjects completed reflux symptom index (RSI) questionnaire,[5] underwent dual channel 24-hour pH/impedance monitoring and flexible laryngoscopy with the evaluation of the reflux finding score (RFS)[6] using the 3.5 mm rhino-pharyngo-laryngofibroscope 11101 RP2 (Karl STORZ). RFS was obtained by one ENT physician. Only subjects that had positive reflux symptom index (RSI > 13) or reflux finding score (RFS > 7) and at least one LPR episode on 24-hour pH/impedance monitoring were further investigated. Being further investigated, patients completed the capsaicin cough challenge (N = 27).

Capsaicin cough challenge was determined using the standardized method of single breath capsaicin inhalation and is in detail described elsewhere.[7] The challenge consists of single breath inhalations of aerosols of the solutions with doubling concentrations of capsaicin. The challenge begins with the inhalation of the capsaicin vehicle (saline) and continues with capsaicin from 0.49 to 1000 μmol/L. The individual doses are separated by 60 s intervals. Aerosols are delivered through the mouth by the inspiration-triggered valve for 500 ms (nasal breathing is prevented by the nasal clip worn throughout the challenge). The coughs are counted during the first 15 s after each dose of capsaicin. Cough reflex sensitivity (cough threshold) is expressed as the lowest concentration of capsaicin causing two coughs (C2) and five coughs (C5). The cough challenge was terminated when patients achieved C5 or the highest concentration of capsaicin was reached (1000 μmol/L). Subjects were instructed to report any sensations and were unaware of the endpoint of the challenge (the number of coughs).

After completing the investigations, patients were prescribed PPI treatment twice daily and were carefully instructed to take PPI regularly, approximately 30 minutes before the breakfast and dinner. After 12 weeks of the PPI treatment, the patients were invited for a follow-up visit. Careful interview was carried out and compliance with the medication was assessed. RSI and RFS were evaluated, capsaicin cough challenge and the dual channel pH/impedance study was performed while still on PPI treatment. Of 27 enrolled patients who were prescribed PPI, 18 patients completed the second capsaicin cough challenge and 24-hour pH/impedance study and were included in the final analysis.

Dual channel 24-hour pH/impedance monitoring was performed as described in our previous study.[8] Briefly, using high resolution manometry (ManoScan ESO High Resolution Manometry System, Medtronic), we located UES and LES and positioned the dual channel pH/impedance catheter so that the proximal pH probe was 1 cm above the UES and the distal pH probe 5 cm above the LES. We used VersaFlex catheters with two pH sensors and eight impedance electrodes (Medtronic), with 3 sizes of catheters (distance 15, 19, and 22 cm between pH sensors). If the exact position of the catheter was not possible to achieve, the catheter of the smaller diameter between the pH sensors was selected and placed according to the UES location. Patients were encouraged to maintain their normal activities, sleep schedule, and eat their usual meals during the 24-hour monitoring and were also asked to mark the time of eating and the horizontal body position on the data recorder (Digitrapper, Medtronic). When performing pH/impedance while "on PPI," the same catheter type has been chosen as in the first measurement, also attention was paid to achieve the same position of the pH sensors. Moreover, patients were instructed to mark the time of taking PPI on the data recorder.

Tracings were visually analyzed using AccuView pH-Z version 5.2 software (Medtronic). The approach to the analysis of pH/impedance tracings and the LPR episodes was described in our previous studies.[8,9] Blinded observer was responsible for evaluation of pH/impedance studies. Meals were excluded from the analysis.

Liquid gastroesophageal reflux episodes were defined as a retrograde 50% drop in impedance starting distally and propagating to at least the next two more proximal measuring segments. Gas gastroesophageal reflux was defined as simultaneous increase in impedance above 5000Ω starting in the most distal impedance channels and propagating to at least the next two more proximal impedance measuring segments. Mixed gastroesophageal reflux (liquid−gas) was defined as gas reflux occurring simultaneously before or during a liquid reflux.

LPR episode was identified when a gastroesophageal reflux episode was temporarily associated with a drop of pH in the proximal pH sensor. Special effort was dedicated to exclude pH changes in the proximal pH sensor due to swallowing, sensor drying and other artifacts. We based our analysis on the assumption that in the absence of swallows a sudden drop of pH in the hypopharynx occurring in a close temporal sequence with a preceding reflux detected by distal pH/impedance is a LPR event. In order to allow for comprehensive analysis of LPR, we performed a separate analysis for proximal pH≤6, 5.5, 5, 4.5, and 4. If pH in the proximal sensor was already lower before the reflux event, the reflux was considered as LPR when pH drop of at least 0.5 unit was observed during the reflux. LPR was quantified as the number of LPR events for each pH and as the pharyngeal acid exposure time (cumulative time of pH ≤ 6, 5.5, 5, 4.5, and 4).

Statistical analysis. The number of reflux episodes is expressed as median and interquartile range (IQR). The duration of reflux episodes is expressed as mean ± SEM. The strength of a linear association between the analyzed variables was measured by the Pearson correlation coefficient (r). Online calculator http://www.socscistatistics.com/tests/pearson/ was used. *P* was calculated based on r and N using online application http://www.socscistatistics.com/pvalues/pearsondistribution.aspx. $P < 0.05$ was considered significant.

RESULTS

Correlation analysis between LPR and coughing

We first tested the hypothesis of cough being *directly stimulated* by the LPR. We determined the reflux burden in the hypopharynx as detected by the proximal (hypopharyngeal) pH probe and performed the correlations between the number of LPR episodes and coughing. As the harmful pH level of the LPR episode is not clearly established, we performed correlations for the whole range of acidity (pH <6, 5.5, 5, 4.5, 4), and determined the amount of hypopharyngeal acidic reflux both as the number of reflux episodes and the hypopharyngeal acid exposure time. Moreover, we performed separate correlations for the number of gas and mixed reflux episodes. Coughing was extrapolated from the RSI questionnaire (RSI question 5—cough after eating and when lying down and question 7—troublesome, bothering cough). For better comprehensibility, cough after eating and when lying down is regarded as Q1 and troublesome/bothering cough is regarded as Q2. The range of values determined by the patient was 0-5.

The number of reflux episodes, cumulative time of acidic reflux in the hypopharynx, and the correlation analysis between the parameters of hypopharyngeal acid burden and the self-reported cough is shown in the Table 1. In-depth

TABLE 1.
Correlation of LPR and Coughing in the Group of Patients that Completed 3 Months Twice Daily PPI Therapy (n = 17)

Number of Refluxes	Median	IQR	Pearson vs. Q1	*P*	Pearson vs. Q2	*P*	Pearson vs. Q1 + Q2	*P*
<4.0	1	[0-1]	0.0943	0.72	0.2895	0.26	0.2049	0.43
<4.5	1	[0-2]	−0.0943	0.72	−0.0877	0.74	0	1
<5.0	1	[1-3]	0.0975	0.71	−0.0775	0.77	0.01	0.97
<5.5	4	[2-6]	02112	0.41	−0.0819	0.76	−0.0624	0.81
<6.0	14	[8-23]	0.118	0.65	0.2112	0.42	0.1459	0.58
Hypopharyngeal Acidic Exposure Time	Mean (sec.)	SEM						
<4.0	3.65	1,58	−0.049	0.85	−0.1364	0.6	−0.0469	0.86
<4.5	15.24	6,23	−0.0906	0.73	−0.2243	0.39	−0.1679	0.52
<5.0	32.76	17,27	−0.2349	0.37	−0.3167	0.22	−0.2941	0.25
<5.5	62.82	21,66	−0.1411	0.59	−0.2486	0.33	−0.2078	0.42
<6.0	239.71	46,36	−0.0574	0.83	0.0265	0.92	−0.0548	0.84
Type of Reflux	Median	IQR						
Gas	2	[0-3]	−0.2415	0.35	−0.404	0.1	−0.3444	0.18
Mixed	3	[2-4]	−0.0447	0.87	−0.1114	0.67	−0.0831	0.75

Amount of LPR is expressed at baseline (off PPI) both as number of refluxes and acidic exposure time.
Correlations using Pearson coefficient are performed. Q1—cough question "Cough After ate and When Lying Down"; Q2−"Troublesome, Bothering Cough" (range 0-5).
Abbreviations: IQR, interquartile range; SEM, standard error of the mean.

analysis of these data could be found in the previous papers that preceded this study.[8] Our comprehensive approach led to no statistically significant relationship between any parameter describing the amount of hypopharyngeal acidic reflux and the self-reported cough (Figure 1). The results suggest against the *direct stimulation* of cough by refluxate in LPR patients.

Correlation analysis between cough reflex sensitivity and coughing

Attempting to further investigate the relationship between LPR and coughing, we explored the possibility that cough is sensitized by reflux. We determined the relationship between the sensitivity of cough reflex and coughing. Cough reflex sensitivity was expressed as the $\log_{10}$ value of the lowest capsaicin concentration that induced 2 coughs (C2) or 5 coughs (C5). Coughing was determined by self-evaluation as described above. Similarly, we found no statistically significant correlation between the sensitivity of cough reflex and coughing (Table 2). To follow up on the investigation of the *sensitization hypothesis*, we proceeded with exploring of the effect of PPI therapy both on coughing and cough reflex sensitivity.

Effect of LPR treatment on the LPR and coughing

The effect of PPI therapy on the acidic LPR was in-detail already presented in our previous study.[9] Briefly, we failed to demonstrate any significant effect of PPI treatment on the decrease of acidic LPR.

We proceeded with the analysis of the effect of PPI treatment on cough itself, as self-reported by the patients (questions Q1 and Q2). In the whole group of 17 patients that completed PPI treatment cough was substantially reduced (approx. by 60%), resulting in the decrease of the "coughing after eating and lying down"—Q1 (2.38 ± 0.5 vs. 0.76 ± 0.34, $P < 0.0001$) and the decrease of the "troublesome and bothering cough"—Q2 (2.45 ± 0.5 vs. 0.37 ± 0.39, $P < 0.01$). Of these 17 patients, we performed further analysis on 11 patients identified as "PPI responders" that achieved normalization of the RSI (RSI $\leq$ 13). These were regarded as having the highest probability of LPR and not only their RSI was substantially reduced (18.36 ± 2.75 vs. 7 ± 1.35, $P < 0.01$) but also the reduction of cough (Q1 and Q2) was numerically more pronounced than in the whole group of 17 patients (2.36 ± 0.63 vs. 0.18 ± 0.12, $P < 0.0001$ and 2.27 ± 0.59 vs. 0.09 ± 0.09, $P < 0.0001$, Q1 and Q2, respectively) (Figure 2A).

Finally we compared the sensitivity of cough reflex after the course of PPI treatment in the PPI responder group (n = 11). Strikingly, no significant reduction of the sensitivity of cough reflex was found in this group of patients regardless of determination of C2 (2 coughs) or C5 (5 coughs) (Figure 2B). The $\log_{10}$ value of the capsaicin concentration inducing C2 was 1.41 ± 0.19 vs. 1.2 ± 0.19 ($P = 0.11$) before and after treatment, respectively. The $\log_{10}$ value of capsaicin concentration inducing C5 was 2.25 ± 0.24 vs. 2.67 ± 0.26 ($P = 0.18$), before and after PPI treatment, respectively.

DISCUSSION

Findings of our study could be summarized as follows: we failed to demonstrate a correlation between the amount of LPR in the hypopharynx and cough and also between cough reflex sensitivity and cough in patients diagnosed with LPR. This is surprising given that PPI therapy led to substantial improvement of cough as evaluated by patients' self-assessment. Our data suggest that neither direct initiation of cough by the LPR (stimulation), nor affection of the cough reflex sensitivity (sensitization) by the LPR is responsible for the initiation of cough in LPR patients. Precise selection of the study population with high probability of LPR further supports our results.

The relationship between gastroesophageal reflux and cough is complex. However, GERD is believed to be one of the leading causes of cough,[10] only minority of GERD patients develop chronic cough.[11] Therefore, establishing cough as a consequence of reflux in an individual is still a challenge. This is particularly true in the LPR population because its diagnosis is challenging with lack of consensus for its verification.[12-14] We partially overcame these obstructions by recruiting the LPR population confirmed by 24-hour dual channel pH/impedance and performing subgroup analyses of patients achieving PPI response and therefore having high probability of LPR (see results—normalization of the RSI).

We first evaluated the possibility that cough is directly initiated by the LPR. This would imply that cough is directly initiated by the stimulation of vagal sensory nerves in the

TABLE 2.
Correlation Between Cough and the Cough Reflex sensitivity

Coughing	Intensity of Cough ±SEM	Pearson vs. C2	*P*	Pearson vs. C5	*P*
Q1	2.375 ± 0.5	−0,2429	0.47	−0,2917	0.39
Q2	2.438 ± 0.5	0,0469	0.89	0,3416	0.18

Analyses are performed in patients off PPI therapy that were regarded as "PPI Responders" (n = 11).
Q1—Cough Question "Cough After ate and When Lying Down"; Q2—"Troublesome, Bothering Cough" (range 0-5); C2 and C5—the Lowest Capsaicin Concentration to Induce 2 Coughs and 5 Coughs, Respectively;
Abbreviation: SEM, standard error of the mean.

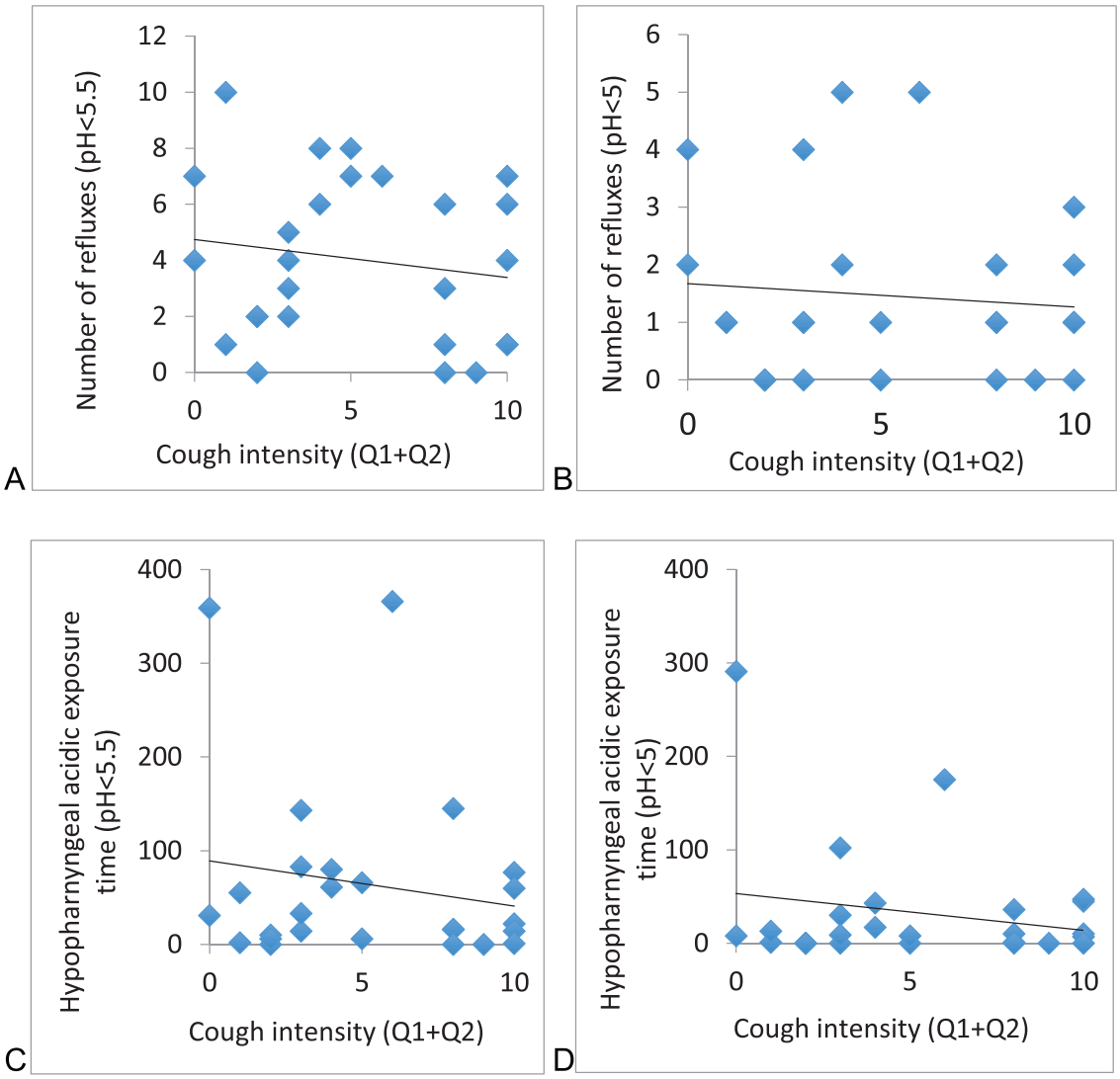

FIGURE 1. Correlation between cough and the reflux burden in the hypopharynx. Cough intensity is expressed as the summation of the self-evaluated intensity of cough. There was no correlation between the cough intensity and the number of LPR episodes that caused the pH drop in the hypopharynx below 5.5 and 5 (**A, C,** respectively, P = NS). By analogy, there was no correlation between the cough intensity and hypopharyngeal acidic exposure time below pH 5.5 and 5 (**B, D**, respectively, P = NS).

larynx and in the upper airways by reflux. One would in this case presume a correlation between the amount of LPR and cough. From the pathophysiological perspective, such correlation would indicate the involvement of thinly myelinated Aδ fibers (abundantly expressed in the larynx or trachea) because Aδ-fiber-mediated cough is triggered by aspirated particles or mucosal acidification.[15] The following mechanisms might theoretically be involved in this direct initiation of cough by reflux: microaspiration of the refluxate into the airways and extension of reflux into the larynx and pharynx.[16] Considered the level of evidence, microaspirations of refluxate into the airways is an unlikely trigger of chronic cough. Studies with broncho-alveolar lavage fluid showed no significant differences between patients with chronic cough and healthy controls.[17] Therefore, the phenomenon of extension of reflux from the pharynx into the larynx and consequent stimulation of the laryngeal Aδ-fibers is explored in this study.

Importantly, although it has been widely accepted that acid is one of the major noxious components of reflux, the amount of refluxate and the level of pH below which the refluxate is noxious for the vagal afferents has not been established.[14,18] Thus, we performed correlations between LPR and coughing for the whole spectrum of pH levels and characterized the hypopharyngeal acid reflux burden both as the number of reflux episodes and as the exposure time. The rationale for sorting the reflux episodes out based on pH was that to the best of our knowledge there are no studies evaluating the amount of acid needed to cause cough (in terms of acidity of the hypopharyngeal pH drop). Although there is no exact pathophysiological explanation for choosing the 0.5 difference in the analyzed refluxes, this was a

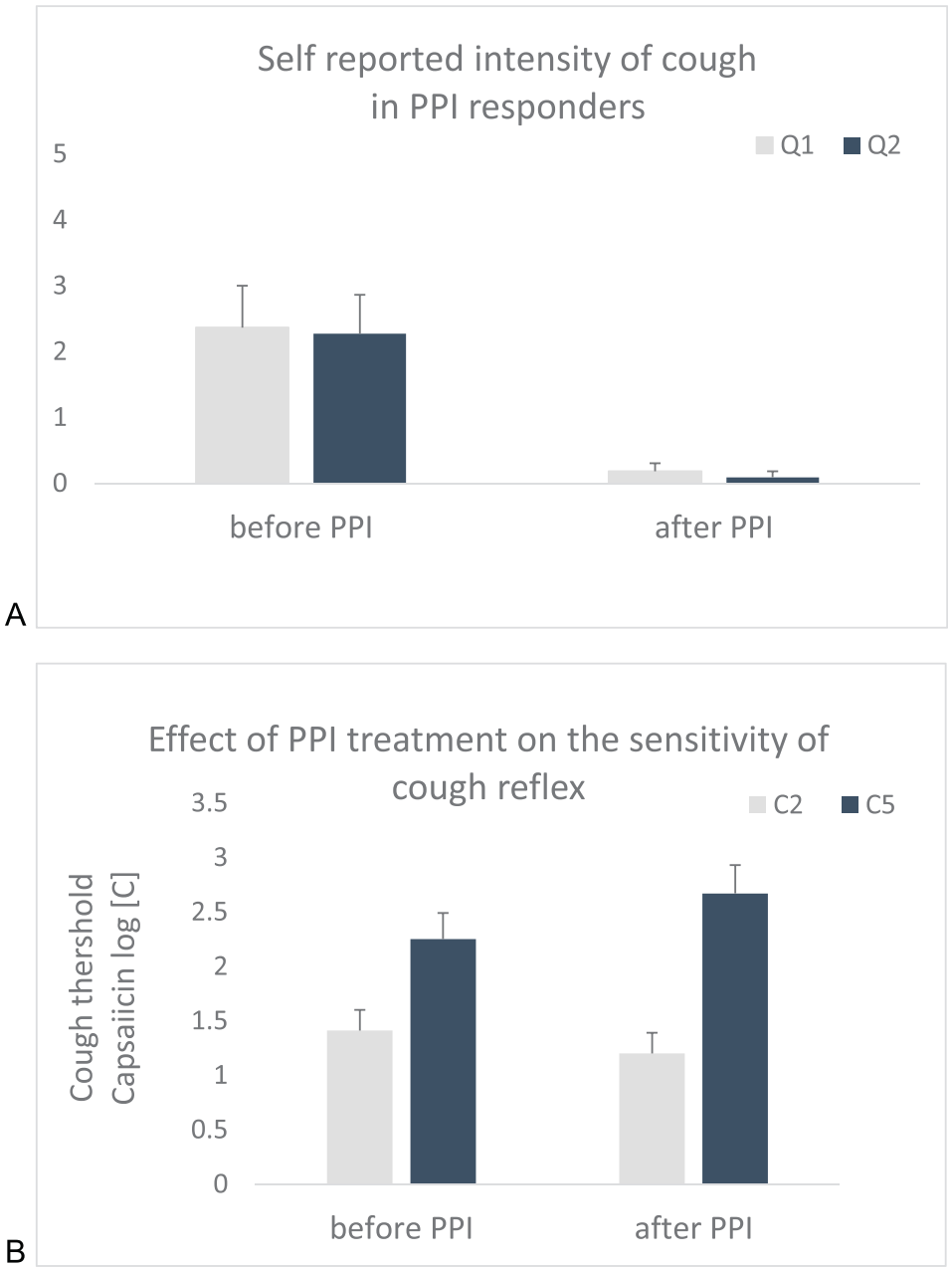

FIGURE 2. Effect of PPI therapy on cough and cough reflex sensitivity. **A**. Significant reduction of cough in the group of PPI responders. Both *cough after eating and when lying down (Q1)* and *troublesome and bothering cough (Q2)* were substantially reduced after 3 months of PPI therapy twice daily (n = 11). **B**. no significant reduction of the lowest concentration of capsaicin inducing 2 or 5 coughs (C2, C5) following the course of 3 months PPI therapy (capsaicin concentration is expressed as $\log_{10}$ value).

rational balance between covering the broad spectrum of acidity of reflux events and avoiding the effort of exhaustive over-analyzing of other categories of refluxes (eg, 0.2 difference in pH). We tried to avoid underestimation of the importance of less frequent but possibly more harmful reflux episodes (eg, pH drop to pH 4 or 4.5) that could get scattered in the more abundant group of refluxes with less prominent pH drop. Despite this comprehensive approach, we failed to find any positive correlation between the amount of LPR and coughing.

Several explanations could be offered for our observations Although acid is the major corrosive component of reflux, other components, eg, pepsin or bile acids that are not readily detected by 24-hour pH/impedance could lead to the stimulation of vagal afferents.[16,19] Detection of their microaspiration is, however, technically challenging and unreliable.[20] Some reports suggest that patients with chronic cough have pepsin levels similar to the healthy controls, which indirectly draws the attention to other noxious stimuli.[21] Another explanation could be that the measurement

lasting 24 hours is unable to detect those reflux episodes that are of pathophysiological importance in the induction of cough and extended measurement might be needed. To follow up on this, presence of acid in the hypopharynx detected by pH/impedance does not necessarily mean that the acid also stimulates nerve afferents in the larynx. Some authors propose the pathophysiological importance of so-called gaseous reflux[22,23] with diagnostic modalities developed for the aerosolized reflux detection.[24,25] Theoretically, this could act as a medium for a wide distribution of reflux particles that enter the peripheral airways. In order to investigate this phenomenon, we performed separate analysis of the gaseous and mixed refluxes, but failed to identify any relationship between their amount per 24 hours and cough (Table 1).

Several studies attempted to explore the relationship between the LPR and cough.[26-28] Their design was somewhat different from ours because they mainly focused on establishing the temporal relationship between reflux episodes and cough. Indeed, in the substantial proportion of chronic cough patients, cough follows reflux more frequently than expected by chance.[16,18,26] Although probably evaluating the same pathophysiological way of initiating cough, our methodology and study population was different —we included LPR patients, not necessarily with pathological acid exposure time and / or number of reflux episodes in the distal esophagus and did not evaluate the temporal reflux-cough episode association, but the correlation between the cumulative amount of hypopharyngeal reflux per 24 hours and the intensity of cough. Our complex approach comprising the analysis of a wide range of acidity and different compositions of refluxate failed to reveal any relationship to the intensity of cough. This indicates that direct initiation of cough by refluxate in LPR patients is improbable.

We proceeded with our study by addressing the hypothesis that sensitization of vagal afferent nerve endings is the mechanism of cough in LPR patients. If this mechanism was involved, cough reflex sensitivity should correlate with coughing and there would exist both improvement of cough and decrease of cough reflex sensitivity after the course of PPI treatment. Confirmation of this hypothesis would indicate the involvement of vagal afferents of unmyelinated C-fibers in the pathogenesis of cough.[29] These are activated by the mediators of inflammation or tissue damage and their activation is somewhat anticipated in LPR patients that commonly express laryngeal inflammation.[2]

In this part of the study, we performed a subgroup analysis of patients that achieved normalization of their symptoms after PPI therapy (RSI < 13), aiming to select patients with the highest probability of LPR. We, however, failed to demonstrate any correlation between the cough reflex sensitivity and coughing (Table 2) (before initiating PPI therapy). Strikingly, although these patients experienced a substantial improvement of coughing (Figure 2A), there was no significant difference in the cough reflex sensitivity on capsaicin cough challenge (Figure 2B), either being expressed as C2 (2 coughs) or C5 (5 coughs).

Sensitization of cough reflex might be initiated by both pulmonary and extrapulmonary sensory fibers.[3,30] The amount of reflux needed to sensitize cough reflex is, unknown. Stimulation of esophageal sensory fibers is involved in cough hypersensitivity. The study from our group showed that esophageal acid infusion sensitizes cough evoked by inhaled capsaicin in patients with GERD and chronic cough.[7] Therefore, reflux itself does not need to reach airways to modulate coughing. On the contrary, our group also showed that PPI therapy had no effect on the cough reflex sensitivity in patients with GERD.[31] The present study focused on reflux episodes with the possibility to reach the hypopharynx and the results suggest against (1) their involvement in the sensitization of cough reflex; (2) involvement of sensitization of cough reflex in LPR patients.

In patients with chronic cough, co-activation of both unmyelinated C-fibers and Aδ fibers induces cough hypersensitivity with both central or peripheral sensitization.[1] Cough hypersensitivity/sensory neuropathy cannot be ruled out in a subgroup of our cohort. Our study, however, was not designed to investigate this phenomenon. Patients with chronic cough as the result of sensory neuropathy experience cough after nontussive stimuli.[32] Indeed, neural plasticity with microstructural changes of afferent nerve endings is involved in cough hypersensitivity, eg, changes in receptors, ion channels, neurochemistry or the fiber density.[29] There is lack of data on the reversibility of cough hypersensitivity in patients with GERD/LPR when PPI treatment is initiated. Taken the available evidence into account, reversibility and/or treatment options of cough hypersensitivity requires further investigation.

CONCLUSION

To conclude, PPI treatment might affect the acidic reflux induced facilitation of cough from the esophagus and might also lead to reversion of laryngeal inflammation mediated C-fiber activation. The extent to which the particular mechanism is affected remains to be established. Our results indicate that the pathophysiology of cough, even in precisely selected group of LPR patients is complex and most probably involves multiple mechanisms. Indeed, this applies not only to the LPR patient group, but also in an individual patient.

Our study has limitations. Firstly, one cannot exclude that the placebo effect of the PPI medication as cough was only self-evaluated. Therefore, although methodologically challenging, placebo-controlled studies or studies with objective cough detection (eg, utilizing cough recorders) might provide even higher quality of evidence of the effect of PPI treatment. Secondly, the number of patients included in the final analysis is rather low. This was mostly due to our effort to select patient of the highest probability of LPR that required adherence to long course of PPI treatment. Finally, self-assessment of cough is necessarily subjective. Objective proof of the improvement of cough would add conclusive evidence of the effect of PPI therapy on cough.

AUTHORS' CONTRIBUTIONS

Martin Ďuriček performed pH/impedance studies, capsaicin cough challenge, data analysis and co-wrote the manuscript. Renata Péčová co-formulated the hypothesis, provided expertise for capsaicin challenge. Peter Bánovčin analysed the data, co-wrote the manuscript. Peter Lipták and Diana Vážanová performed pH/impedance studies and analysed the data.

CONFLICTS OF INTEREST

No conflicts of interest declared.

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