

# Dilated intercellular space in chronic laryngitis and gastro-oesophageal reflux disease: at baseline and post-lansoprazole therapy

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## SUMMARY

### Background

Dilation of intercellular spaces is reported to be an early morphological marker in gastro-oesophageal reflux. It remains unknown if this marker is useful in diagnosing reflux-related chronic laryngitis.

### Aim

To determine histopathology and electron microscopic changes in oesophageal and laryngeal epithelium in chronic laryngitis.

### Methods

In this prospective blinded study, we enrolled 53 participants: 15 controls, 20 patients with GERD and 18 patients with chronic laryngitis. The latter two groups were subsequently treated with lansoprazole 30 mg bid for 12-weeks. Baseline and postacid suppressive therapy biopsies were obtained from distal oesophagus and laryngeal postcricoid areas. Biopsy specimens were evaluated for histopathology and dilated intercellular space changes.

### Results

There was no significant increase in oesophageal or laryngeal epithelium intercellular spaces among GERD or laryngitis patients compared with controls at baseline or postacid suppressive therapy. Only patients with GERD had significantly ( $P = 0.03$ ) higher proportion of moderate-to-severe oesophageal spongiosis and basal cell hyperplasia, which normalized postacid suppressive therapy.

### Conclusions

There was no increase in the width of intercellular spaces in the oesophagus or larynx in GERD or chronic laryngitis at baseline or postacid suppressive therapy. Our findings question the uniform presence of dilated intercellular space in patients with GERD.

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## INTRODUCTION

Gastro-oesophageal reflux disease (GERD) is a common condition affecting up to 30% of the US population.<sup>1-3</sup> Patients with GERD often present with typical symptoms of heartburn and regurgitation; however, some may present atypically with asthma, chronic cough or chronic laryngitis.<sup>4</sup> Management of the latter group of patients is challenging given the multi-factorial nature of the symptom complex. As a consequence, acid suppressive therapy with proven efficacy in patients with typical symptoms of GERD is less efficacious in those with chronic laryngitis.<sup>5, 6</sup> It is estimated that up to 10% of chronic laryngitis patients presenting to an otolaryngologist do so because of complaints that are suspected GERD-related.<sup>7</sup> However, the correct diagnosis of reflux disease in this group of patients is problematic due to lack of specificity of laryngoscopic evaluation and poor sensitivity of oesophagoscopy and ambulatory pH monitoring.<sup>8-10</sup> Laryngoscopic signs originally thought to be GERD-related may be present in healthy asymptomatic volunteers.<sup>9</sup> Oesophagoscopy most often reveals normal appearing mucosa and pH monitoring is normal in more than 50% of this patient group.<sup>10</sup> Thus, a more sensitive diagnostic tool is needed for patients with chronic laryngitis.

The presence of dilated intercellular spaces in the oesophageal mucosal biopsy is suggested as a sensitive marker of GERD in patients with oesophagitis<sup>11, 12</sup> or in those with non-erosive reflux disease.<sup>11-13</sup> In the original study by Tobey *et al.*,<sup>11</sup> oesophageal biopsy specimens from patients with GERD had significantly wider intercellular spaces compared with controls. This observation was based on animal studies suggesting an increase in paracellular permeability of oesophageal lumen due to exposure to acid and pepsin<sup>14</sup> as well as weakly acidic material.<sup>15</sup> Subsequent studies have also suggested reversibility of the dilated intercellular spaces postacid suppressive therapy.<sup>16</sup>

Given a lack of an objective diagnostic tool in patients with chronic laryngitis, we hypothesized that the presence of dilated intercellular spaces in the oesophageal or larynx mucosa would help diagnose objectively patients whose symptoms may be GERD-related. Thus, in this prospective blinded study, we aimed (i) to determine if light microscopy and/or transmission electronic microscopy findings could differentiate control from patients with GERD and those with suspected GERD-related chronic laryngitis; and (ii) to evaluate if tissue changes, if any, are predictors of response to acid suppressive therapy.

## MATERIAL AND METHODS

The study was performed in accordance with the Declaration of Helsinki, Good Clinical practice and applicable regulatory requirements. The Vanderbilt Institutional Review Board approved this clinical trial (NCT00373997 and NCT00444145). Each patient signed a consent form prior to any study-related procedures.

### Participant selection

We conducted a prospective blinded longitudinal study to determine clinical significance of dilated intercellular spaces in three groups of participants: controls, gastro-oesophageal reflux disease and chronic laryngitis suspected reflux-related. The control group consisted of asymptomatic subjects with no prior history of gastro-oesophageal reflux disease defined as heartburn and/or regurgitation; they had no previous therapy with acid suppressive medication such as proton pump inhibitors, histamine receptor antagonists or antacids; they did not have previous history of oesophagitis or prior surgical therapy for gastro-oesophageal reflux disease. They underwent oesophagogastroduodenoscopy (EGD) for reasons other than reflux disease, which included anaemia or diarrhoea. The participants with GERD comprised patients with a history of heartburn and/or regurgitation as the chief complaint, as well as objective presence of either erosive oesophagitis at endoscopy (Los Angeles Classification) or abnormal pH monitoring off acid suppressive therapy. Those with Barrett's oesophagus were excluded. The third group consisted of participants with a clinical diagnosis of chronic laryngitis based on persistent throat symptoms of cough, throat clearing, hoarseness or sore throat and laryngoscopic findings of irritation in the posterior larynx considered reflux-related. This group was referred by the Vanderbilt Voice Center or the Allergy, Sinus and Asthma Program. Participants with prior ear, nose or throat or gastrointestinal surgery, cancer and/or radiotherapy or patients presenting with major oesophageal motility disorders, or previous history of alcoholism were excluded.

### Study design

All participants completed a detailed questionnaire assessing current and past medical history, current medication as well as information on subject demographics (age, gender, and race), presence, severity and frequency of GERD (heartburn/regurgitation) and extra-oesophageal symptoms (cough, hoarseness, throat clearing, sore throat, globus sensation, heartburn, regurgitation, problem swallowing, chest pain and discomfort to talk),

history of smoking, history of alcohol use and presence of voice/throat and nose symptoms. Subsequently, all participants underwent EGD (conscious sedation) with biopsies (regular forceps; 2.4 mm, cold Captura, Cook medical, Bloomington, IN, USA) at least 7 days off proton pump inhibitors or 4 days off histamine receptor antagonists. Two sets of two biopsies were taken from each participant, one set from the distal oesophagus and one set from the larynx. Oesophageal biopsies were taken from 5 cm above the gastro-oesophageal junction. Laryngeal biopsies were obtained from the postcricoid area. One set of each oesophageal and laryngeal biopsy was sent for histopathology evaluation and the other set was sent for transmission electron microscopic analysis. Both pathologists were blinded to endoscopic findings, participants' demographics, group classification and results of pH monitoring. They were not blinded to the oesophageal or laryngeal location of the biopsies.

Subsequently, patients with GERD and those with chronic laryngitis were invited to participate in a subsequent phase of the study and to take lansoprazole 30 mg twice daily for 12 weeks. After 12 weeks, those who agreed to participate underwent a second EGD with biopsies from the same oesophageal and laryngeal locations as the pre-therapy protocol. Oesophageal and laryngeal biopsies were again assessed for histopathology and electron microscopic changes. Also, at this time, patients scored the chief complaint response to acid suppressive therapy on an analogue horizontal scale from 0% (no improvement or symptoms worse) to 100% (complete response). Prior to the second EGD, assessments were made for drug accountability, concomitant medication use and the presence of potential medication side effects. Changes in life style modification were not discussed or implemented during the study period.

#### Histopathology evaluation

Specimens for histopathology evaluation were immediately fixed in formalin after EGD and then embedded in paraffin. Serial sections for 4- $\mu$ m thickness were cut and stained with haematoxylin-eosin. The histopathology grading was based on the modified Chadwick scoring system.<sup>17</sup> For the oesophagus and the larynx, intraepithelial lymphocytes were scored as 0 (few, within normal limits); 1 (slightly increased over normal); 2 (moderate increase); 3 (marked increase). Eosinophils and neutrophils were counted separately per high power field in the area of most dense infiltrate. Basal layer hyperplasia was scored as 0 (normal), 1 (20–30% of epithelial thickness), or 2 (>30% of epithelial thickness). Ulcers were

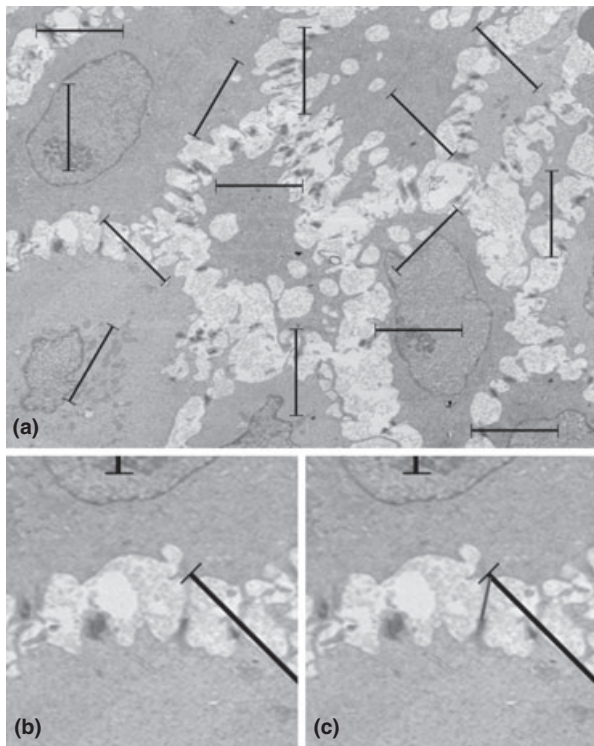
scored as absent or present. Basal layer spongiosis was scored as 0 (absent), 1 (mild), 2 (moderate), or 3 (marked). For the oesophagus, papillary elongation was scored as normal, or increased and extending greater than 50% of epithelial thickness. An overall injury grade was assigned to biopsies based on the following features: Normal: no or slight papillary elongation and no intraepithelial lymphocytes, eosinophils or neutrophils; Mild: 1+ basal layer hyperplasia and/or 1–4 eosinophils, with or without papillary elongation; Moderate: 2+ basal layer hyperplasia and/or 5–19 eosinophils per high power field; Severe: ulcer, neutrophils and/or 20 or more eosinophils per high power field.

#### Transmission electron microscopy and morphometric analysis

Specimens for electron microscopy evaluations were immediately fixed in 2.5% glutaraldehyde rinsed in cacodylate buffer (0.1 M) and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer. Following fixation, tissues were dehydrated through a graded series of ethanols to 100% and embedded in Spur resin. Thick sections (0.5  $\mu$ m) were made and stained with toluidine blue for light microscopic examination to determine suitable regions for the sectioning. Areas showing cross section through the oesophageal or laryngeal epithelium and lumen were chosen for electron microscopy examination. Thin sections (~80 nm) were cut of these regions and examined using an FEI CM12 transmission electron microscope operated at 80 kilo-electron volts. To quantify the distance between epithelial cells, 10 arbitrarily selected epithelial regions were examined for each sample at a magnification of 4400 $\times$ . The same randomly computerized grid of 14 lines was superimposed on each area of interest for analysis (Figure 1a). Each time one of the 14 lines crossed a portion of the cell membrane (Figure 1b), a transect was drawn between the crossing point and the closest cell wall of an adjacent cell (Figure 1c). An average of 10 transects were digitally measured per field. A total of 102 gaps were computer measured for each sample and the lowest and highest values were discarded. The analysis was performed on the supra-basal region for both tissues.

#### Oesophageal pH testing

Ambulatory pH monitoring was performed for 48 h using a wireless pH monitoring device (Given Imaging Inc., Duluth, GA, USA). Study patients were instructed to stop taking all proton pump inhibitors for 7 days and H2RA's for 4 days before undergoing evaluation.



**Figure 1** | An example of the transmission electron microscopy measurement technique. (a) 14 computer-generated lines were superimposed to the area of interest. (b) Close up of an area where one of the 14 lines is crossing a cell wall for measurement of intercellular space. (c) A transect was drawn between the crossing point and the closest cell wall of an adjacent cell and distance between cells was measured.

Wireless capsules were calibrated by submersion in buffer solutions at pH 7.0 and pH 1.0 and then activated by magnet removal. Patients underwent EGD with conscious IV sedation for visual anatomic inspection and distance measurements from the incisors to the squamocolumnar junction. Capsules were then placed using the manufacturer's delivery system at 6 cm above the squamocolumnar junction, and attached with vacuum suction of 600 mm Hg. Capsule placement was confirmed at endoscopy. After successful placement, patients were given wireless pH recorders to wear about their waists or to keep within 3–5 feet at all times. Recording devices received pH data sampling transmitted by the capsule at 433 Hz with 6-s sampling intervals. Patients were instructed to perform their normal daily activities and dietary practices. Distal oesophageal pH recording was conducted for a total of 48 h. During this time, patients kept diaries of meal times, symptoms and supine

position. After completion of the 48-h study, data were downloaded from the recording devices to dedicated computers using Datalink software (Given Imaging Inc.). Patient diary information was manually entered into the computer-based record. Measurements of the total, upright, and supine percentage time when oesophageal pH was below 4 were determined over day 1 and day 2 of the wireless study. Acid exposure time (% time pH < 4) greater than 5.3% per day was considered abnormal.<sup>18</sup>

#### Data and statistics

Data were collected and stored at the secure web-based Vanderbilt Digestive Disease Center REDCap (Research Electronic Data Capture) (1 UL1 RR024975 NCR/NIH). REDCap is an application designed to support data capture for research studies providing (i) an intuitive interface for validate data entry; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for seamless data downloads to common statistical packages; and (iv) procedures for importing data from external sources. There was strict control and supervision of the data entry and access for this study.

Sample sizes were based on prior publications showing increased intercellular dilation in GERD compared to controls.<sup>13, 15, 16, 19, 20</sup> Using the reported variability from previous studies ( $\sigma^2 = 0.2$ ) and the observed sample sizes from our two smallest groups ( $n = 15$  and  $n = 17$ ), we had 80% power to detect a 0.19 unit difference in average intracellular space among the control and chronic laryngitis groups. We had larger power for any other pairwise comparisons of interest. Power calculations were based on a 2-sample *t*-test with the average intracellular space in a subject as the outcome of interest.

Reported results were obtained by using linear regression to compare intercellular spaces across groups defined by health status (control, GERD, chronic laryngitis), presence of oesophagitis, site and therapy. Regression models were fit using generalized estimating equations with robust standard errors that accounted for the correlation arising from taking repeated EM measurements on the same subject. The repeated measures for intercellular spaces are summarized using the mean ( $\pm$ SD) as no notable outliers were found that would heavily impact the average for any subject. Demographic data reported as median (interquartile) or % subjects affected where appropriate and Pearson's chi-Square and Wilcoxon rank sum tests were used to compare groups.

## RESULTS

## Demographics

Oesophageal and laryngeal biopsies were obtained from 53 participants: 15 controls, 20 patients with GERD and 18 patients with chronic laryngitis. Demographic data for the study participants are summarized in Table 1. No significant differences were found among the groups in terms of age, gender, race, and alcohol or tobacco use. Oesophageal mucosa was normal in the control population. 13/20 (65%) of patients with GERD had erosive oesophagitis at baseline (LA grade A = 38%, B = 23%, C = 0% and D = 38%) and the remaining 7/20 (35%) had abnormal oesophageal pH monitoring at baseline. 3/18 (17%) patients with chronic laryngitis had erosive oesophagitis at baseline (LA grade A = 100%) and 6/18 (33%) had abnormal oesophageal pH monitoring. Nineteen participants (10 patients with GERD and nine patients with chronic

laryngitis) underwent treatment with twice daily lansoprazole 30 mg for 12 weeks followed by a second EGD with oesophageal and laryngeal biopsies. At follow-up endoscopic evaluation erosive oesophagitis was healed in all 19 patients. Median (interquartile) symptomatic response to acid suppressive therapy was achieved in 90% (83–90%) of patients with GERD and 65% (8–90%) of patients with chronic laryngitis. No serious adverse events occurred during the study. Six patients complained of cramping, constipation, diarrhoea, bloating or headaches which were considered to be related to PPI use. None stopped the medication because of these side effects.

## Histopathology

There was no difference in baseline oesophageal histology grade among the three study groups with respect to intraepithelial lymphocytosis (IELs), eosinophil, or polymorphonuclear (PMN) cell counts, papillary elongation or presence of ulcers (Table S1). However, as compared with the controls or patients with chronic laryngitis, patients with GERD had significantly ( $P = 0.03$ ) higher proportion of moderate-to-severe oesophageal spongiosis and basal cell hyperplasia (Table S1). Baseline laryngeal pathology was not different among the three groups (Table S2). Baseline oesophageal and laryngeal histology revealed predominately normal or mild overall injury grades among the three study groups (Table 2). Only oesophageal biopsies in patients with GERD had significantly ( $P = 0.03$ ) more overall injury compared with the other two study groups. Postacid suppressive therapy, oesophageal spongiosis, basal cell hyperplasia and overall injury scores improved for patients with GERD (Tables 2 and S1). There was no difference in overall injury scores postacid suppressive therapy between groups (Table 2).

**Table 1** | Demographic data by participant groups

Demographics	Participant groups		
	Control, n = 15	GERD, n = 20	Laryngitis, n = 18
Age (years)*	39 (31–48)	50 (42–56)	51 (46–59)
Gender (% female)	67	50	72
Race (% Caucasian)	93	90	89
Tobacco status (% non-smoker)	87	85	89
Alcohol status (% non-drinker)	60	60	56

\* Median (interquartile).

**Table 2** | Oesophageal and laryngeal histopathology by participant groups at baseline and 12-weeks postacid suppressive therapy

Biopsy site	Overall injury grade	Baseline evaluation				Post-therapy		
		Normal, n = 15	GERD, n = 20	Laryngitis, n = 18	P	GERD, n = 10	Laryngitis, n = 9	P
Oesophagus	Normal/mild	93	70	100	0.03	100	89	0.5
	Moderate/severe	7	30	0		0	11	
Larynx	Normal/mild	93	90	90	0.9	100	100	1.0
	Moderate/severe	7	10	10		0	0	

Results are expressed as percentage of participants with normal/mild vs. moderate/severe histology grades.

### Transmission electron microscopy and morphometric analysis

Measurements by the electron microscopist blinded to patient category found no significant increase in oesophageal or laryngeal intercellular spaces between GERD or laryngitis groups compared with controls at baseline and postacid suppressive therapy (Table 3). Examples of transmission electron microscopy for each group at oesophageal and laryngeal sites are shown in Figure 2. The mean ( $\pm$ SD) intercellular space for patients with chronic laryngitis was less in the distal oesophagus than for patients with GERD. There was great variability and marked overlap of measured intercellular spaces for each study group at both biopsy sites at baseline and postacid suppressive therapy (Figure 3). In patients with GERD or those with chronic laryngitis, the mean ( $\pm$ SD) intercellular space measurements were not affected by oesophagitis status of patients (Figure 4).

### DISCUSSION

In this prospective blinded study, we did not demonstrate an increase in intercellular spaces of the distal oesophagus or larynx among patients with GERD or those with chronic laryngitis compared with controls at baseline or postacid suppressive therapy. Additionally, we found that the histopathological features of oesophageal and laryngeal tissue were no different among patients with chronic laryngitis than controls. However, as expected, patients with GERD were more likely to have moderate-to-severe injury in the distal oesophageal histology at baseline which was corrected by acid suppressive therapy.

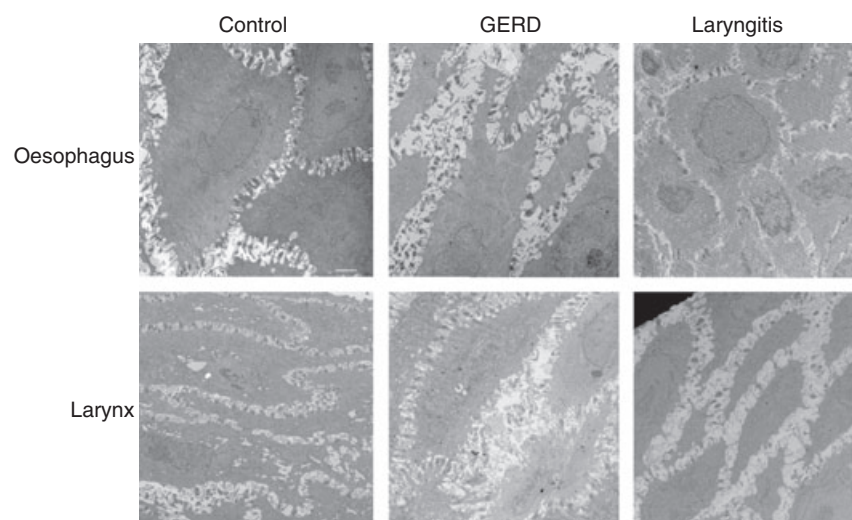
Our study findings question the uniform presence of dilated intercellular spaces in patients with GERD and suggest that this objective measure may not be present in all patients. Several possibilities may explain the divergence of our data from prior studies.<sup>11-13, 16, 19</sup> First, the circumferential and proximal extent of gastroduodenal

**Table 3** | Mean ( $\pm$ s.d.) of intercellular space average over 100 sampled locations for each group and at both biopsy sites

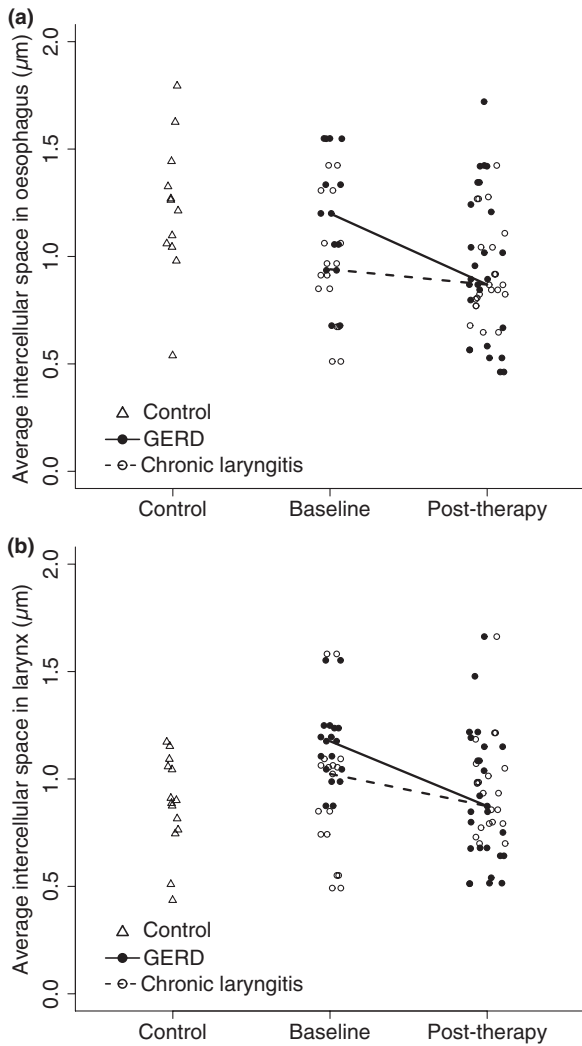
Biopsy site	Baseline evaluation				Post-therapy		
	Control, <i>n</i> = 15	GERD, <i>n</i> = 20	Laryngitis, <i>n</i> = 18	<i>P</i> *	GERD, <i>n</i> = 10	Laryngitis, <i>n</i> = 9	<i>P</i>
Oesophagus	1.20 $\pm$ 0.32	1.03 $\pm$ 0.36	0.86 $\pm$ 0.19	0.001	1.20 $\pm$ 0.31	0.96 $\pm$ 0.19	N.S.
Larynx	0.88 $\pm$ 0.22	0.94 $\pm$ 0.35	0.91 $\pm$ 0.20	N.S.	1.16 $\pm$ 0.19	0.93 $\pm$ 0.33	N.S.

There was no significant increase in intercellular spaces in GERD or laryngitis patients compared with controls and no difference among the groups postacid suppressive therapy.

\* Significantly less in laryngitis vs. controls; N.S. implies not significant.

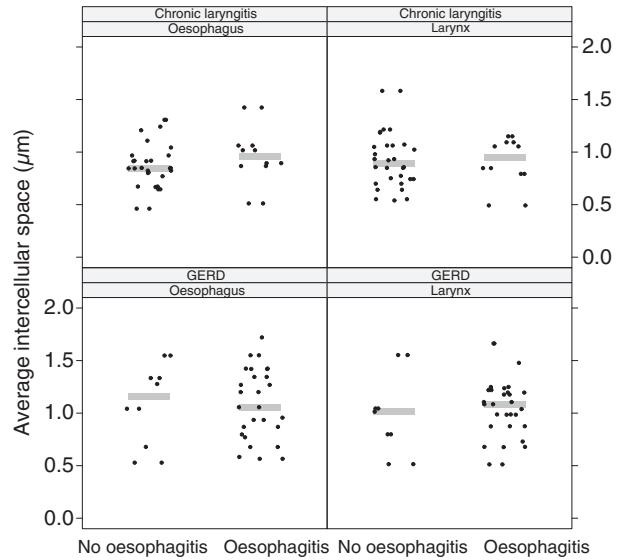


**Figure 2** | Examples of oesophagus and larynx transmission electron microscopy for controls, patients with GERD and those with chronic laryngitis.



**Figure 3** | Individual transmission electron microscopy measurements for oesophagus (a) and larynx (b) for controls, patients with GERD and those with chronic laryngitis. Points represent the intercellular space averaged over 100 sampled locations for each subject. Lines depict the change in the median of the average intercellular space from baseline to post-therapy.

reflux contents varies along the oesophageal lumen among GERD patients.<sup>20, 21</sup> Thus, random biopsies at different oesophageal locations may or may not reveal the presence of intercellular space dilation. Location of the distal oesophageal biopsies in the prior studies have varied ranging from zero to 5 cm above the gastro-oesophageal junction.<sup>12, 13, 16, 19–25</sup> Intercellular space dilation can vary significantly even within a small distance of 1-cm lateral<sup>21</sup> and 1–2 cm above<sup>20</sup> endoscopic oesophagitis. A second reason for the difference in our data may be due to variability of prior studies in



**Figure 4** | Individual transmission electron microscopy measurements for oesophagus and larynx for patients with GERD and those with chronic laryngitis by oesophagitis status. Points represent the intercellular space averaged over 100 sample locations for an individual subject, and bars indicate the median of the average intercellular space for each group.

reporting blinding of pathologists to subject groups which may have biased their study outcome in favour of presence of intercellular space dilation.<sup>22, 26</sup> Third, prior reports have varied on the mucosal layers at which the dilated intercellular spaces were measured; some used the luminal layers,<sup>12, 13, 24, 25</sup> whereas others measured the basal layers.<sup>16, 19, 23, 26</sup> Fourth, there is heterogeneity in measurement techniques as well as location of the selected areas to assess DIS which explains the different values for DIS reported in the literature.<sup>11, 13, 19</sup> Finally, it is possible that dilated intercellular space is not a specific marker for GERD alone. More recent studies have suggested the presence of this condition with *Candida* infection, food allergies,<sup>27</sup> hypertonic solutions<sup>28</sup> and even psychological stress.<sup>29</sup> Strengths of the current study include uniformity of biopsy protocol, blinding of the evaluating pathologist and electron microscopist to patient groups and lack of change in group baseline intercellular space measurements with acid suppressive therapy. Thus, dilated intercellular spaces may not be sensitive or specific markers of GERD, but our findings have provided an impetus for future studies to examine this pathophysiological process closely, especially with respect to geographical variability in the distal oesophagus.

Patients with chronic laryngitis are often diagnosed with GERD without objective criteria.<sup>8</sup> This overzealous and often inappropriate diagnosis has led to many unnecessary tests and treatments by otolaryngologists, gastroenterologists and primary care physicians. We have previously shown that laryngoscopy is a nonspecific marker for the diagnosis of reflux in patients with chronic laryngitis.<sup>9</sup> Oesophagoscopy and pH monitoring, commonly performed in this group of patients, are often normal and suffer from poor sensitivity.<sup>8, 10, 30</sup> The clinical practice of empiric trial with proton pump inhibitors has not stood the rigour of controlled studies.<sup>5, 6</sup> Our data in this study do not support the use of intercellular space measurements as an objective means of diagnosing GERD in patients with chronic laryngitis. The intercellular space measurements in our study were not higher between patients with chronic laryngitis or those with GERD compared with controls at baseline or postacid suppressive therapy. Although the measurements in patients with chronic laryngitis were significantly less than those for controls, the direction of the change is in the direction opposite to what is clinically relevant and it may represent the variability in intercellular space measurements highlighted above. To date, two studies have reported using transmission electron microscopy in patients with chronic laryngitis.<sup>26, 31</sup> Amin *et al.* reported dilated intercellular spaces in 20 patients with laryngitis compared with five controls. Although the pathologists were blinded to subject groups, the evaluation of the intercellular spaces was subjective without objective measurements.<sup>31</sup> Franchi *et al.* reported objectively wider intercellular spaces in 15 patients compared with seven controls; however, there were no details provided regarding blinding of the pathologists.<sup>26</sup> In addition, unlike our study, neither of the above two studies included oesophageal biopsies or patients with GERD as comparators. Therefore, the findings from our study add to the wealth of evidence against reflux as the cause in many patients initially so suspected.

Our study has several limitations: First, the sample size in each patient group is small; however, it is similar to if not larger than most prior reports in the area of intercellular space microscopy, which had shown increased intercellular spaces between GERD and controls;<sup>11-13, 16, 19</sup> thus, a type II error is less likely. Second, the participants in our control group were not healthy volunteers, but patients with conditions other than GERD. Thus, silent reflux could not be excluded in this group of patients. However, as the intercellular space

measurements for this group as well as those with GERD were similar at baseline, and more importantly postacid suppressive therapy, the values most likely represent true baseline measurements. Finally, in our study, we obtained oesophageal biopsies at 5 cm above the gastro-oesophageal junction in all patients for the purpose of protocol standardization. It is possible that the purported intercellular oesophageal changes may have been present, but in a more distal location closer to the stomach; however, exploring this possibility was beyond the scope of our protocol and the location of 5 cm above the GE junction is again similar to most other studies in this area.<sup>11-13, 16, 19</sup> Future studies are needed to determine the graded variation in distal oesophageal intercellular space in GERD patients.

In summary, the present study does not demonstrate an increase in intercellular space of the distal oesophagus or larynx among patients with GERD or those with chronic laryngitis compared with controls at baseline or postacid suppressive therapy. Our data question the uniform presence of dilated intercellular space in patients with GERD and suggest a possible graded distal oesophageal variation of this pathophysiologic marker. We do not support the use of intercellular space measurements as objective means of diagnosing GERD in patients with chronic laryngitis.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Oesophageal histopathology by participant groups at baseline and 12-weeks postacid suppressive therapy. Results are expressed as percentage of participants.

**Table S2.** Laryngeal histopathology by participant groups at baseline and 12-weeks postacid suppressive therapy. Results are expressed as percentage of participants.



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