Laryngopharyngeal Reflux and Voice Disorders: A Multifactorial Model of Etiology and Pathophysiology

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Summary: Objective. The aim of this paper is to shed light on the pathogenesis and pathophysiological mechanisms underlying the development of hoarseness related to laryngopharyngeal reflux disease (LPRD).
Material and methods. PubMed, Embase, and The Cochrane Library were searched for the terms reflux, laryngopharyngeal, laryngitis, voice, and hoarseness. Experimental and clinical studies providing substantial information about the occurrence of voice disorders, laryngeal histologic changes, or any pathophysiological processes related to LPRD were included by two independent investigators.
Results. Of the 104 studies reviewed, 47 studies that met our inclusion criteria were analyzed. LPRD leads to significant macroscopic and microscopic histopathologic changes in the mucosa of the vibratory margin of the vocal folds. More and more studies suspect that epithelial cell dehiscence, microtraumas, inflammatory infiltrates, Reinke space dryness, mucosal drying, and epithelial thickening are probably responsible for the hoarseness related to reflux and the impairment of the subjective and objective voice quality evaluations.
Conclusion. Future clinical studies examining the pathophysiology of hoarseness related to LPRD should take into consideration all potential mechanisms involved in the development of hoarseness.
Key Words: Voice–Laryngopharyngeal–Reflux–Hoarseness–Pathophysiology.

INTRODUCTION

Laryngopharyngeal reflux disease (LPRD) is an inflammatory condition defined as the backflow of gastric contents into the laryngopharynx, where it comes in contact with the tissues of the upper aerodigestive tract.[1] LPRD occurs in 4%–30% of patients who visit otolaryngology departments and up to 55% of patients with hoarseness.[2–4] LPRD is characterized by chronic inflammation of the laryngopharynx and, more broadly, the tissues of the upper aerodigestive tract.[5] Patients with LPRD usually complain of a myriad of nonspecific symptoms including throat clearing, persistent cough, heartburn, globus sensation, or hoarseness, with hoarseness accounting for 71%–79% of the symptoms reported.[6,7] Historically, LPRD has often been given as a default diagnosis for hoarseness. However, current beliefs would suggest that although LPR may coexist with other vocal fold disorders, other vocal fold pathologies are often diagnosed via laryngovideostroboscopy, which might explain the hoarseness. In addition, some data showed that the major etiologic factor for hoarseness more than 3 months in duration is LPRD, because LPR occurs in 55%%–79% of patients with resistant hoarseness.[8,9] Among the laryngostroboscopic findings, vocal fold edema has often been suggested as the main factor affecting the vocal fold vibrations, leading to hoarseness,[10,11] but recent data call this assumption into question, especially for mild and moderate LPRD where there is no or mild edema.[3,12,13] To date, the precise mechanisms of voice disorders related to LPRD remain incompletely understood.

This systematic review was designed to shed light on the etiology, pathogenesis, and pathophysiological mechanisms underlying the development of hoarseness related to LPRD and to identify the laryngostroboscopic findings associated with hoarseness related to LPRD.

MATERIALS AND METHODS

Literature search

We conducted a systematic literature research on PubMed, Embase, and The Cochrane Library databases to identify experimental and clinical studies directly or indirectly related to the development of hoarseness associated with LPRD. This research covers different aspects of LPRD and hoarseness including pathogenesis, basic science, pathophysiology, genetic, and biomolecular studies. The keywords used were "reflux," "laryngopharyngeal," "laryngitis," "voice," and "hoarseness." When data were found in more than one publication, we used the data reported in the largest and most recent publications. This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist for reviews and meta-analysis[14] and the Participant, Intervention, Comparison, Outcome, and Study design criteria for the clinical studies (Table 1). The local ethics committee approved this review.

Types of studies

The following inclusion criteria were used: prospective, controlled or uncontrolled, clinical, or experimental studies published since 1996, which was the year of the first paper that identified LPRD as a different entity from gastroesophageal reflux disease.[16]

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TABLE 1.
Participant, Intervention, Comparison, Outcome, and Study Design (PICOS) Criteria Used for the Clinical Studies Composing This Systematic Review[15]

Parameters	Inclusion Criteria	Exclusion Criteria
Patients	Adults ≥18 years with suspected LPRD The confirmation of the diagnosis required at least: (1) signs and symptoms± (2) pH metry confirmation± (3) Peptest confirmation± (4) a 3- or 6-month empirical therapeutic response	Patients under 18 years of age
Intervention	Medical± Diet and behavioral advice± Surgery	
Comparator	(1) pre- to post-treatment comparisons ± controlled group or (2) case-controlled studies (at baseline) with healthy subjects (control group)	
Outcomes	(1) laryngostroboscopic findings± (2) aerodynamic measurements± (3) acoustic parameters± (4) electroglottography findings	
Study design	Randomized controlled trials Nonrandomized controlled trials Prospective or retrospective studies Cross-sectional studies	Case reports

Only the probative findings were extracted from the included studies, especially those that conveyed direct or indirect information on vocal fold mucosa function. We determined the grade of recommendation for each clinical study following the Oxford Centre for Evidence-Based Medicine evidence levels.[17] We classified the experimental research according to the topic of the study, which was the involvement of LPRD in the defense mechanisms of the mucosa or in the inflammatory reaction.

Data extraction

All references were sorted manually to extract all descriptions of subjects meeting the diagnosis of laryngopharyngeal reflux by the first author (JRL). Each study was identified based on PubMed abstracts, available full text, title, or keywords that made reference to LPRD. The author (JRL) was not blinded to the papers' authors, their institutions, or the journal of publication.

RESULTS

Experimental studies

The database search yielded 34 articles. A total of 24 papers were included and represented 17 controlled and seven uncontrolled studies. Fifteen studies used human laryngeal samples, and nine were based on animal models (Tables 2 and 3). The studies that examined the inflammatory reactions of the laryngeal mucosa (N = 14) are shown in Table 2. The studies that focused mainly on the defense mechanisms of the laryngeal mucosa (N = 7) are described in Table 3.

Clinical studies

Our initial PubMed, Cochrane Library, and Embase searches identified 70 articles. From these, we included 23 relevant papers for a total of 1342 patients (Tables 4 and 5). Of these studies, we reported five controlled studies that assessed objective voice quality at baseline for a total of 485 patients (Table 5). Of the prospective trials, we selected 10 uncontrolled, 6 controlled, and 2 randomized placebo-controlled trials, which accounted for 857 patients with LPRD (Table 5). The flowchart showing the process of article selection is described in Figure 1.

DISCUSSION AND EVIDENCE SYNTHESIS

Experimental studies

Etiology, pathogenesis, and chronic inflammatory reaction

Previous studies have shown that irritation of the laryngeal mucosa in LPRD is due to two mechanisms. The main mechanism concerns the direct effect of the gastric content reflux (ie, acid, pepsin, trypsin, bile salts, and some gastroduodenal proteins) on the laryngeal mucosa (Table 2)[32,54,65,66]; the second mechanism (indirect effect), which remains controversial, involves the mucosa chemoreceptor stimulation resulting from refluxate from the stomach in the distal portion of the esophagus, with vagal reflexes followed by coughing and throat clearing.[67–69] The current literature tends to confirm with high prevalence the direct effect of gastric content, but to date, the existence of an indirect effect has not been excluded and could add to the first theory.

Indeed, most human and animal studies have demonstrated the presence of pepsin in extra-[30,70] and intracellular[20,21,24,32] laryngeal structures, which suggests a key role in the inflammatory process (Table 2). Pepsin may be active to some degree at any pH between 1.5 and 6.0, although a longer exposure time may be necessary at pH 5 to produce lesions.[22,37,71] Interestingly, the inactivated pepsin molecules in the laryngeal epithelium have

TABLE 2.
Research Studying the Inflammatory Reaction of the Laryngeal Mucosa (Human and Animal Samples)

References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Adhami et al 2004[18]	Prospective controlled	N.P.	Canine laryngeal samples Exposed to Gr1: LPR (N = 42) Bile ± pepsin ± trypsin acid 3 times per week (4 w) Gr2: CT (N = 4) Saline solution t0 = 0, t1 = 4w	Microscopy	Histologic infl. score Intraepithelial inflammation squamous metaplasia and erosion ulcers, stromal inflammation periglandular inflammation fibrosis	 Gr1 > Gr2 Gr1 > Gr2 Gr1 > Gr2 Gr1 > Gr2 Gr1 > Gr2
Cohen et al 2004[19]	Prospective controlled	N.P.	Canine laryngeal samples Gr1: Injured vocal folds (N = 3) exposed to pepsin pH 2 or pH 6 every day for 12 days Gr2: Injured vocal folds (N = 3) exposed to saline solution Gr3: Uninjured vocal folds (N = 2)	Microscopy	Histologic analysis Cellular infiltrate Fibronectin Procollagen I	 Gr1 = Gr2 > Gr3 Gr1 = Gr2 > Gr3 Gr1 = Gr2 > Gr3
Johnston et al 2004[20]	Prospective controlled	pH metry (double probe)	Human laryngeal samples Vocal folds (a) ventricles (b) Gr1: LPR (N = 9) Gr2: CT (N = 12)	IHC and Western blotting	Pepsin tissue level correlation Pepsin-CA III depletion	Gr1 > Gr2 (a, b) + (a, b)
Gill et al 2005[21]	Prospective controlled	Clinical diagnosis RSI > 11 + RFS > 5 or pH metry findings	Human laryngeal samples Vocal folds (a) Posterior commissure (b) ventricles (c) Gr1: LPR (N = 18) Gr2: CT (N = 12)	IHC and Western blotting	Intracellular pepsin E-cadherin CA III Correlations Pepsin and lack of CA III	Gr1 > Gr2 (a, b, c) Gr2 > Gr1 (a, b, c) Gr2 > Gr1 (a, c) S (a)
Ylitalo and Thibeault 2006[22]	Prospective uncontrolled	N.P.	Human laryngeal samples ventricles (a) Posterior commissure (b) exposed to pepsin + pH 4 or 5 during 10, 30, 60, 240 s	RT-PCR	Messenger RNA expression ventricles fibroblasts TGFβ-1, VEGF FGF-2 ATF-3 CTGF, MMP1 MMP-2, Decorin, EGR-1 Messenger RNA expression Post. com. fibroblasts TGFβ-1, FGF-2, CTGF, MMP1 EGR-1 ATF-3, VEGF MMP-2, Decorin	pH-pepsin-time effect S, NS, NS NS, NS, NS S, S, S S, NS, S S, NS, NS pH-pepsin-time effect S, NS, S NS, NS, NS S, NS, NS NS, NS, S

(*Continued*)

TABLE 2.
(*Continued*)

References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Franchi et al 2007[23]	Prospective controlled	1. Laryngeal sympt. 2. Posterior laryngitis 3. Esophageal pH metry (GERD)	Human laryngeal samples Posterior commissure Gr1: LPR patient biopsies (N = 15) Gr2: Healthy (N = 7)	Microscopy	Intercellular spaces dilatation Epithelial cell	Gr1 > Gr2
Johnston et al 2007[24]	Prospective uncontrolled	N.P.	Human laryngeal samples Exposed to human pepsin Posterior commissure (N = 2) Posterior cricoid area (N = 2)	Microscopy	Presence of Intracellular pepsin	 +
Rees et al 2008[25]	Prospective controlled	Clinical diagnosis RSI > 21	Human laryngeal samples Posterior vocal folds tissue Gr1: LPR (N = 12) Gr2: CT (N = 11)	IHC	Cell infiltration B cells, neutrophils, eosinophils, monocytic CD8 lymphocytes (luminal and basal layers) Expression of MHC I, II β2-microglobulin (deepest layers)	 Gr1 = Gr2 Gr1 = Gr2 Gr1 > Gr2 Gr1 = Gr2 Gr1 > Gr2
Reichel et al 2008[26]	Prospective uncontrolled	pH metry (double probe) or clinical diagnosis	Human laryngeal samples Vocal folds Posterior commissure Gr1: pH metry LPR (N = 14) Gr2: Clinical LPR (N = 7)	IHC	Expression E-Cadherin β-Catenin	 Gr2 > Gr1 Gr1 = Gr2
Shimazu et al 2009[27]	Prospective controlled	N.P.	Rat laryngeal samples Posterior commissure Gr1: GERD rats (N = 26) Gr2: CT rats (N = N.A.) t0 = 0, t1 = 8 w, 16 w	Microscopy	Changes in laryngeal tissues Mucosal thickening Capillaries Proliferation and dilatation	 + +
Johnston et al 2010[28]	Prospective controlled	Clinical diagnosis RSI 21–30	Human laryngeal samples LPR patients (N = 12) Healthy (N = 11)	IHC	Expression CD161 MHC I, II MHC β2m MHC CD1d	 Gr1 > Gr2 Gr1 = Gr2 Gr1 > Gr2 Gr1 > Gr2
Vaezi et al 2010[29]	Prospective controlled	Clinically LPR	Human laryngeal samples Posterior commissure Gr1: LPR (N = 18) Gr2: GERD (N = 20) Gr3: CT (N = 15)	Microscopy	Cell counts Intraepithelial lymphocytosis Eosinophil Polymorphonuclear Laryngeal intercellular spaces Basal cell laryngeal hyperplasia	 Gr1 = Gr2 = Gr3 Gr1 = Gr2 = Gr3 Gr1 = Gr2 = Gr3 Gr1 = Gr2 = Gr3 Gr1 = Gr2 = Gr3

(*Continued*)

TABLE 2.
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References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Bulmer et al 2010[30]	Prospective uncontrolled	N.P.	Porcine laryngeal samples ventricles (a) Vocal folds (b) Posterior commissure (c) Supraglottic (d) Subglottic (e) Incubated (pH 2, 4 ± pepsin)	Optical density (solution absorbance)	Tissue damages pH 2 Ventricles Vocal folds Posterior commissure Supraglottic Subglottic Tissue damages pH 4 Ventricles Vocal folds Posterior commissure Supraglottic Subglottic Tissue damages pH 7.4 Ventricles Vocal folds Posterior commissure Supraglottic Subglottic	pepsin–/pepsin+ –/+ –/+ –/– +/+ +/+ –/+ –/– –/– –/+ +/+ – – – – –
Erickson and Sivasankar 2010[31]	Prospective controlled	N.P.	Porcine laryngeal samples (N = 52) Exposed to Gr1: pH 3 ± porcine pepsin Gr2: pH 7 ± porcine pepsin t0 = 0, t1 = 15 min, t2 = 30 min	Voltage clamp Microscopy	Transepithelial R of VF with acidic pepsin Transepithelial R of VF with acid-only Histologic changes (t2) Epithelial shedding Epithelial intracellular edema Epithelial extracellular edema Basilar edema Sub-basilar edema Vacuolization	t0 > t1 > t2 (Gr1) t0 = t1 = t2 (Gr2) t2: Gr1 > Gr2 Gr1 and 2: t0 > t2* Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2
Jiang et al 2011[32]	Prospective controlled	pH metry (double probe)	Human laryngeal samples Posterior commissure Gr1: acid and nonacid LPR (N = 7a, 8na) Gr2: CT (N = 21)	IHC	Intracellular pepsin	Gr1 > Gr2 Gr1a = Gr1na

(*Continued*)

TABLE 2.
(*Continued*)

References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Hu et al 2013[33]	Prospective controlled	N.P.	Rabbit laryngeal samples Gr1: Rabbit reflux (N = 4) 12-week reflux episodes Gr2 : CT (N = 4)	Microscopy	Vocal cords Intercellular space Lymphocytes infiltration	 Gr1 > Gr2 Gr1 > Gr2
Durkes and Sivasankar 2015[34]	Prospective controlled	N.P.	Porcine laryngeal samples Incubated (acid + pepsin) (3 per week [4 w]) Gr1 = reflux pigs (N = 4) Gr2 = CT pigs (N = 4)	Microscopy	Vocal fold morphology Collagen structures Elastin structures Epithelial cellularity Lamina propria cell infiltrate Ultrastructural alterations EISD Micro-ridge height	 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2
				RT-PCR	Gene expression E-cadherin Zona occludens-1 CFTR Epithelial Na+ Channel IL-1β, TNFα, Ifγ	 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2 Gr1 = Gr2

Note: Activating transcription factor 3.
* Reversibility effect.
Abbreviations: CA, carbonic anhydrase; CFTR, cystic fibrosis transmembrane conductance regulator; CT, control; CTGF, connective tissue growth factor; EGR-1, epidermal growth factor 1; EISD, epithelium intercellular spaces diameter; FGF-2, fibroblast growth factor 2; GERD, gastroesophageal reflux disease; Gr, group; IHC, immunohistochemistry; IL, interleukin; LPR, laryngopharyngeal reflux; MHC, major histocompatibility complex; MMP, matrix metalloproteinase 1; N, number; N.P., not provided; NS, not significant; RFS, reflux finding score; RSI, reflux symptom index; RT-PCR, reverse transcription polymerase chain reaction; S, significant; Sympt., symptoms; t, time; TGFβ-1, tumor growth factor β-1; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VF, vocal fold; w, week.

TABLE 3.
Research Studying the Defense Mechanism of the Laryngeal Mucosa

References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Axford et al 2001[35]	Prospective uncontrolled	N.P.	Human laryngeal samples Vocal folds (a) Posterior commissure (b) LPR Patients (N = 9)	IHC (1) Western blot (2)	Expression CA I (a, b) CA II (a, b) CA III (a, b)	(1 and 2) +/+ +/+ –/+
Johnston et al 2003[36]	Prospective controlled	N.P.	Human laryngeal samples (N = 78) Vocal folds (a) Posterior commissure (b) Ventricule (c) Gr1: LPR (N = 26) Gr2: CT (N = 19) Porcine laryngeal mucosa Exposed to Acid stress (20 min) pH 2 and pH 4 t0 = 0, t1 = 20m post	Western blotting and IHC and *in situ* hybridization	Gene expression CA I Vocal folds Posterior commissure Ventricules CA II CA III Vocal folds Posterior commissure Ventricules Correlation post. com. CA III level—sympt. E-cadherin expression Laryngeal tissues (a, b, c) MUC 4 and 5AC expression Vocal folds Posterior commissure Ventricules *In vitro* expression CA I (pH 2 and pH 4) CA III (pH 2 and pH 4)	 Gr1 = Gr2 23/25 25/26 24/24 Gr1 = Gr2 Gr2 > Gr1 9/24 20/25 15/24 S 20/51 (37%) Gr2 > Gr1 Gr2 > Gr1 Gr2 > Gr1 t0 = t1 t0 > t1 (reversible)
Johnston et al 2007[37]	Prospective uncontrolled	N.P.	Porcine laryngeal cells Incubated (pH 1.5–6.5 + pepsin) during 20 min	Western blotting	Depletion CAIII and Sep70 pH 1.5 pH 2.0, 2.5 pH 3.0, 3.5, 4.0 pH 4.5, 5.0, 6.0 pH 6.5	 ++ +++ ++ + –
Samuels and Johnston 2008[38]	Prospective controlled	Clinical diagnosis RSI and RFS	Human laryngeal samples Posterior commissure Gr1: LPR (N = 3) Gr2: CT (N = 2)	RT-PCR	Mucin gene expression MUC2, 3, 5AC, 5B,	 Gr2 > Gr1

(*Continued*)

TABLE 3.
(*Continued*)

References	Design	LPR Diagnosis	Sample or Patient Characteristics	Analysis	Outcomes	Results
Erickson-Levendoski 2012[39]	Prospective uncontrolled	N.P.	Porcine laryngeal samples (N = 57) Exposed to Gr1: pH 3 ± porcine pepsin t0 = 0, t1 = 60 min	Electrophysiological techniques	Ions transport (bicarbonate)	t1 > t0
Ali et al 2014[40]	Prospective controlled	N.P.	Human laryngeal samples (N = 27)	*In situ* hybridization	Gene expression (LPR)	
			Healthy Laryngeal biopsies (N = 3)		MUC 1, 2, 4	CT = LPR (a, b, c)
			Ventricules (a)		MUC3	CT = LPR (a, c)
			Posterior commissure (b)			CT > LPR (b)
			Vocal folds (c)		MUC5AC	CT > LPR (a, b)
						CT = LPR (c)
					Gene expression (CT)	
					MUC1, 3, 4	+ (a, b, c)
					MUC2, 5AC	+ (a, c)
Min et al 2016[41]	Prospective controlled	Clinical diagnosis RSI > 13 and RFS > 7	Human laryngeal samples Posterior commissure Gr1: LPR (N = 10) Gr2: CT (N = 18)	IHC	Expression of	
					CA III	Gr2 > Gr1
					Hsp70	Gr2 > Gr1

Abbreviations: CA, carbonic anhydrase; CT, control; CTGF, connective tissue growth factor; Gr, group; Hsp, heat shock protein; IHC, immunohistochemistry; LPR, laryngopharyngeal reflux; N, number; N.P., not provided; NS, not significant; Post. Comm., posterior commissure; RFS, reflux finding score; RSI, reflux symptom index; RT-PCR, reverse transcription polymerase chain reaction; S, significant; Sympt., symptoms; t, time; w, week.

TABLE 4.
Clinical Case-controlled Studies

References	Design	EBM	LPR Diagnosis	Patient Characteristics	Outcomes	Results (Better Values)
Ross et al 1998[42]	Monocentric controlled study	IIB	Presence of: (1) signs, (2) symptoms of LPR	N = 69 LPRP, 20 CT Gr I: patients with positive result in pH metry Gr II: patients with negative result in pH metry Gr III: patients without pH metry Gr IV: control group	F0 male and female Jitter (%) Shimmer (%)	Gr I, II, III = CT Gr I, II, III = CT CT > Gr I, II, III
Pribuisiene et al 2006[43]	Monocentric controlled study	IIB	Presence of (1) signs (2) symptoms of LPR (3) 1 and 2 for at least 3 months (4) esophagitis	N = 108 LPRP, 90 CT Gr I: LPR patients Gr II: control group	Videolaryngostroboscopy %Jitter, %Shimmer, NNE F0 Maximum phonation time	CT > Gr I CT > Gr I Gr I = CT CT > Gr I
Oguz et al 2007[44]	Monocentric controlled study	IIB	Presence of: (1) signs (2) symptoms of LPR	N = 48 LPRP, 64 CT Gr I: objective LPR patients Gr II: LPR symptomatic Gr III: control group	Jitter (local, absolute, rap, ppq) Shimmer (local, dB, apq3, 5, 11) F0 (male and female), NHR	CT > Gr I = II Gr I = II = CT Gr I = II = CT
Kumar and Bhat 2008[45]	Monocentric controlled study	IIB	Presence of: 24-h pH metry	N = 30 LPRP, 30 CT Gr I: LPR patients (30) Gr II: control group (CT, 30)	Vital capacity Mean airflow rate Maximum phonation duration Phonation quotient	Gr II > Gr I Gr I = Gr II Gr II > Gr I Gr I = Gr II
Akyildiz et al 2012[46]	Monocentric controlled study	IIB	Presence of: (1) signs, (2) symptoms of LPR (RSI > 13)	N = 230 LPRP, 48 CT Group I: LPR patients Group II: control group	Voice turbulence index %Jitter and NHR %Shimmer	CT > Gr I (F, M) CT > Gr I (F) Gr I = CT (M) CT > Gr I (F, M)

Abbreviations: APQ, amplitude perturbation quotient; CT, control; F0, fundamental frequency; Gr, group; LPRP, laryngopharyngeal reflux patients; M , month; MPT, maximum phonation time; N, number; NHR, noise-to-harmonic ratio; NNE, normalized noise energy; NP, not provided; NS, not significant; PPQ, pitch perturbation quotient; RFS, reflux finding score; RSI, reflux symptom index; S, significant.

TABLE 5.
Prospective Studies

References	Design	E	LPR Diagnosis	Patient Characteristics	Treatment Voice Outcomes	Results	Treatment Type	DT
Shaw and Searl 1997[47]	Prospective uncontrolled	IIB	Presence of: (1) signs (2) symptoms of LPR	N = 96 LPRP	Laryngoscopic grading system Patients with initial hoarseness Jitter, shimmer, F0, frequency range	S (except granuloma) S, S, S, S	1. Omeprazole 20 mg b.i.d. 2. Gaviscon 30 mL 3. Cisapride 10 mg q.i.d.	12
Habermann et al 1999[48]	Prospective uncontrolled	IIB	Presence of: (1) signs (2) symptoms of LPR (3) voice disorders	N = 29 LPRP	Videostroboscopy findings Mucosa aspect, reddening Posterior commissure Vocal process Vocal folds Ventricles Quantity of mucus	 S S S S S S	Pantoprazole 40 mg 1/d	6
Hamdan et al 2001[49]	Prospective uncontrolled	IIB	Presence of: (1) signs (2) symptoms of LPR (3) confirmed GERD	N = 22 LPRP	F0, RAP, %Shimmer, NHR, VTI Maximum phonation time	NS NS	1. Pantoprazole 40 mg b.i.d. 2. Cisapride 20 mg b.i.d.	4
Noordzij et al 2001[50]	Prospective randomized controlled	IB	Presence of (1) signs, (2) symptoms of LPR (3) 24-h pH metry	Gr 1 = LPRP (N = 15) Gr 2 = CT (N = 15)	Vocal fold edema Arytenoid redness Arytenoid edema Interarytenoid irregularity Mucus accumulation	NS, NS; Grl = GrII (placebo) NS, NS; Grl = GrII (placebo) NS, NS; Grl = GrII (placebo) NS, NS; Grl = GrII (placebo) NS, NS; Grl = GrII (placebo)	Grl: Omeprazole 40 mg b.i.d. Gr II: Placebo b.i.d.	8
Selby et al 2003[51]	Prospective uncontrolled	IIB	Presence of (1) signs, (2) symptoms of LPR	N = 13 LPRP	HNR Jitter (%) Shimmer (%)	S NS NS	1. Omeprazole 40 mg/d or Lansoprazole 30 mg/d 2. Speech therapy during 8 w	8–10

(*Continued*)

TABLE 5.
(*Continued*)

References	Design	E	LPR Diagnosis	Patient Characteristics	Treatment Voice Outcomes	Results	Treatment Type	DT
Siupsinskiene et al 2007[52]	Prospective controlled	IIA	Presence of	N = 120 LPRP, 113 CT	After 1–2 weeks		Gr I: 100	5
			(1) signs,	Gr I = LPR (N = 120)	Posterior laryngitis score	Gr I: S ; Gr II: NS, Gr III: NS	IA: 20 mg once a day	
			(2) symptoms of LPR	IA = 28	(edema, redness, nodularity)		IB: 20 mg b.i.d.	
			(3) 1 and 2: at least 1 mo	IB = 48	Vocal Dysfunction Degree	Gr I: S ; Gr II: NS, Gr III: NS	IC: >40 mg per day	
				IC = 24	(F0 and intensity ranges, high F range, VRP parameters)		Gr II: diet	
				Gr II = LPR (N = 20)			Gr III: CT group	
				Gr III = CT (N = 113)	After 4–5 weeks			
					Posterior laryngitis score	GrI: S; GrII: NS; GrIII: S		
					Vocal dysfunction degree	GrI: S; GrII: NS; GrIII: S		
Williams et al 2004[53]	Prospective uncontrolled	IIB	Presence of (1) signs, (2) symptoms of LPR (3) 1 and 2: at least 3 mo	N = 20	Laryngoscopic findings (erythema, edema, ulceration, subglottic, glottic lesions)	S	Omeprazole 20 mg t.i.b.	12
Sereg-Bahar et al 2005[54]	Prospective uncontrolled	IIB	Presence of (1) signs, (2) LPR symptoms (RSI)	N = 43 LPRP	F0, Jitter, Shimmer, NHR RFS	NS, NS, S, S S	Esomeprazole 40 mg	8
Ogut et al 2007[55]	Prospective uncontrolled	IIB	Presence of (1) esophagitis (2) RSI > 14	N = 38 LPRP	RFS %Jitter, %Shimmer, NHR, PPQ, APQ	S S, S, S, S, S	Nissen fundoplication Laparoscopic	27–36 after sur.
Jin et al 2008[3]	Prospective uncontrolled	IIB	Presence of (1) signs, (2) symptoms of LPR (3) 24-h pH metry	N = 40	RFS (2e, 4e, 8e, 12e, 16e, and 20e s)	NS, S, S, S, S, NS	1. Lansoprazole 30 mg 1/d	20
					Jitter (% ; 1–2m ; 3–4m)	S, S	2. Levosulpiride 25 mg/d	
					Shimmer (% ; 1–2m ; 3–4m)	S, S	3. or Mosapride 5 mg 3/d	
					HNR (1–2m ; 3–4m)	S, S		

(*Continued*)

TABLE 5.
(*Continued*)

References	Design	E	LPR Diagnosis	Patient Characteristics	Treatment Voice Outcomes	Results	Treatment Type	DT
Sala et al 2008[56]	Prospective uncontrolled	IIB	Presence of	N = 22 LPRP	Erythema and edema of vocal folds	S, S	1. Omeprazole 20 mg/d or Pantoprazole 40 mg/d	PPI:
			(1) signs,		Erythema and edema of pharynx	S, S	Lansoprazole 30 mg/d at least 6 months	12
			(2) symptoms of LPR		Erythema of hypopharynx	S		Sur.
			(3) voice disorders		Edema of hypopharynx	NS		54
							2. Nissen fundoplication only in nonresponders	
Vashani et al 2010[57]	Prospective controlled	IIA	Presence of	N = 32 LPRP	Jitter (Gr A, Gr B)	S, S	Gr1 :	6
			(1) signs,	Gr 1 = LPR (N = 16)	Shimmer (Gr A, Gr B)	S, NS	1. Omeprazole 20 mg b.i.d.	
			(2) symptoms of LPR	Gr 2 = LPR (N = 16)	NNE (Gr A, Gr B)	S, S	2. voice therapy 2 b.i.w.	
			(3) hoarseness		HNR (Gr A, Gr B)	S, NS	Gr2 : 1. Placebo b.i.d.	
Fass et al 2010[58]	Prospective randomized controlled	IB	Presence of	N = 41 LPRP	Videostroboscopy (RFS)	Gr I = Gr II	Gr1: esomeprazole 20 mg b.i.d.	12
			(1) signs,	Gr I = LPR (N = 24)	pitch range	Gr I = Gr II		
			(2) symptoms of LPR	Gr 2 = LPR (N = 17)	sustain vowel frequency	Gr I = Gr II	Gr 2: placebo b.i.d.	
					sustain vowel intensity	Gr I = Gr II		
					sentence frequency	Gr I = Gr II		
					sentence intensity	Gr I = Gr II		
Ayazi et al 2012[59]	Prospective controlled	IIA	Presence of	N = 32 GERDP, 55 CT	Electroglottography		Fundoplication	12
			(1) signs,	Gr 1 = GERD (N = 32)	Frequencies irregularity (CFx)	Gr I > Gr II		
			(2) symptoms of LPR	Gr 2 = CT (N = 55)	Amplitude irregularity (CAx)	Gr I > Gr II		
			(3) 24-h pH metry		Closed-phase ratio irregularity (CQx)	Gr I = Gr II		
Park et al 2012[60]	Prospective controlled	IIA	RSI > 13 + RFS > 7	N = 100 LPRP	RFS (change in score ≥3)	Gr I < Gr II (3 m)	Gr 1:	12
				Gr 1 = LPR (N = 50)	Jitter	GrI = GrII (1, 2, and 3m)	1. Omeprazole 20 mg b.i.d.	
				Gr 2 = LPR (N = 50)	Shimmer	GrI = GrII (1, 2, and 3m)	Gt 2:	
							1. Omeprazole 20 mg b.i.d. 2. voice therapy 1/w	

(*Continued*)

TABLE 5.
(*Continued*)

References	Design	E	LPR Diagnosis	Patient Characteristics	Treatment Voice Outcomes	Results	Treatment Type	DT
Wan et al 2014[61]	Prospective controlled	IIA	RSI > 13 + RFS > 7 or pH monitoring	N = 58 LPRP, 58 CT	RFS tot and all categories*	Gr1: S, Gr2: S	Esomeprazole 20 mg b.i.d.	4
				Gr 1 = RSI/ RFS diagnosis	Jitter, Shimmer, HNR	Gr1: S, Gr2: S		
				N = 29	F0	Gr1: NS, Gr2: NS		
				Gr 2 = pH metry diagnosis	MPT	Gr1: NS, Gr2: NS		
				N = 29				
				Gr 3 = CT (N = 58)				
Sahin et al 2015[62]	Prospective controlled	IIA	Presence of	N = 41 LPRP, 26 GERD	RFS	Gr I : S ; Gr II : S ; Gr I > Gr II	1. Laparoscopic surgery	108
			(1) heartburn or acid regurgitation, or both	Gr 1: LPR	F0	Gr I = Gr II	2. If symptoms after surgery: PPIs	
				Gr 2: GERD	Sound pressure level	Gr I = Gr II		
			(2) RSI > 13 and RFS > 7 (GrI)		%Jitter, %Shimmer, NHR, APQ, PPQ	Gr I = Gr II		
			(3) RSI < 13 and RFS < 7 (GrII)		Maximum phonation time	Gr I = Gr II		
Lechien et al 2016[64]	Prospective uncontrolled	IIB	RSI > 13 + RFS > 7	N = 80 LPRP	RFS	Gr1: S, Gr1A: S, Gr1B: S	(1) Pantoprazole 20 mg b.i.d.	12
				Gr1: LPR (N = 80)	Granulation or nodularities posterior	Gr1B > Gr1A		
				Gr1A: LPR Cured (N = 58)	Tongue tonsils hypertrophy	Gr1B > Gr1A		
				Gr1B: LPR Resistant (N = 22)	MPT*, PQ	Gr1: S, Gr1A: NS, Gr1B: S*		
					%Jitter, RAP, PPQ	Gr1: S, Gr1A: S, Gr1B: NS		
					%Shimmer, APQ	Gr1: S, Gr1A: S, Gr1B: S		
					PFR, STD, vF0, vAm	Gr1: S, Gr1A: S, Gr1B: NS		

* Except granuloma; posterior commissure hypertrophy (pH group).

Abbreviations: APQ, amplitude perturbation quotient; b.i.d., twice a day; CT, control; d, daily; DBT, diet and behavioral treatment; DT, duration of treatment (weeks); E, evidence-based level; F0, fundamental frequency; GERD, gastro-esophageal disease; Gr, group; HNR, harmonic-to-noise ratio; LPRP, laryngopharyngeal reflux patients; M, month; MPT, maximum phonation time; N, number; NHR, noise-to-harmonic ratio; NNE, normalized noise energy; NP, not provided; NS, not significant; PFR, phonatory F0 range; PPQ, pitch perturbation quotient; PQ, phonatory quotient; RAP, relative average perturbation; RFS, reflux finding score; RSI, reflux symptom index; S, significant; STD, standard deviation of F0; vAm, peak amplitude variation; vF0, frequency variation; VTI, voice turbulence index; w, weeks.

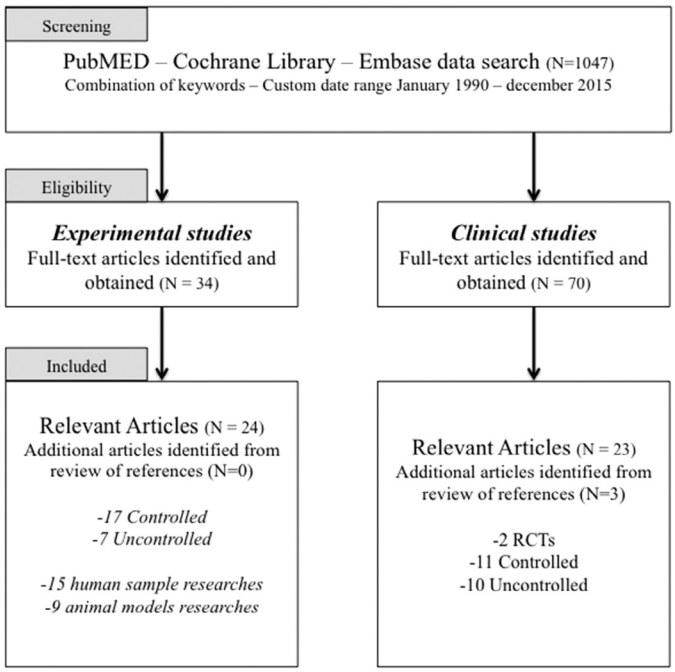

FIGURE 1. The flowchart shows the process of article selection for this study.

good stability and persist over time. Thus, the pepsin reactivation may be mediated by the next reflux gastric episode (extracellular pepsin) or once the molecule is endocytosed in intracellular compartments at a lower pH (such as the Golgi apparatus), which leads to intracellular injuries. The first way describing the negative intermittent activity of pepsin under certain conditions might explain the presence of intermittent complaints of LPRD following only weakly acidic reflux, and the second may involve the resistance of some patients to the empirical treatment.[24] Pepsin endocytosis causes mitochondrial damage and promotes the expression of many genes involved in the recruitment of inflammatory cells, migration, differentiation, growth, and angiogenesis.[22,24,28] The changes in the transcript levels of these genes, which occur in the cells of the vocal folds,[19,30] appear to be dependent on acid exposure time, suggesting the importance of considering not only the occurrence of a reflux episode but also the duration of the episode.[18,22,25,28,30,33] In addition, acidic pepsin also negatively alters expression of growth factors involved in wound repair, angiogenesis, and vasculogenesis.[72,73] It is important to keep in mind that pepsin may act individually or in combination with biliary salts. This combination must not be underestimated because biliary reflux is an important cause of LPRD acid suppressive therapy resistance and vocal fold lesions,[74] which may occur in 35%–40% of patients.[75]

Laryngeal defense mechanisms

Many factors predisposing the laryngeal mucosa to injury have been described in the current literature (Table 6). In addition, LPRD-related stress is known to affect the mucosal defense mechanisms, favoring epithelial injuries. These mechanisms include carbonic anhydrase (CA), heat shock proteins, mucin, and trefoil peptide expression (Table 3).

The first altered mechanism concerns the pH-regulating effect of CA in the laryngeal mucosa.[35] On one hand, it has been shown in a porcine model that the acid and pepsin stress may reversibly and acutely increase bicarbonate production by laryngeal cells, which decreases cell membrane transepithelial resistance.[31,39] This acute adaptive response to mucosal injury seems to be mediated by the intracellular CA isoform III,[36] which plays a key role in the neutralization of refluxed gastric acid and results in reduced peptic activity. On the other hand, some evidence has demonstrated that chronic acidic pepsin can reduce the expression of CA III in vocal folds and ventricular tissues in both human[20,36,41] and animal[37] laryngeal samples, especially at pH levels of 1.5 and 3.0.[37] From a clinical standpoint, Gill et al and Johnston et al observed a positive correlation between the pres-

TABLE 6.
Defense Mechanism and Favoring Factors of LPRD

	Laryngeal	Esophageal
Defense mechanisms	CA	Esophageal motility
	Mucus	LSO and SSO tonus
	Laryngeal proton pump*	
	Natural laryngeal pH (pH > 7.0)	
	Genetic pattern†	
Favoring factors	Laryngeal topographic localization and the proximity with SSO	LSO insufficiency
		Esophageal heterotopic gastric mucosa
	Pseudostratified epithelium	
	Lower acid clearance of the mucosa	
	Laryngeal proton pump*	
	Genetic pattern†	

* Some evidence suggests a defense role[76] and other consider as pejorative favoring factor.[77]
† The composition of the vocal fold and the expression of the defense mechanisms are influenced by the genetic pattern that constitutes simultaneously a defense or favoring factors.
Abbreviation: CA, carbonic anhydrase.

ence of pepsin and the lack of CA III expression in vocal folds and ventricular tissues.[20,21]

The second protective mechanism involves the mucus of the luminal surfaces of the epithelium, especially the mucins, which serve as a selective physical barrier between the extracellular environment and the epithelial cells, and ensures the hydration and lubrication of the vocal fold surfaces.[78,79] The hydration of mucins determines the volume of the mucus gel that contributes to the rheological properties and mediates binding and sequestration of a range of host defense factors. Particular attention should be paid to this point because aberrant expression of mucin genes may underlie several inflammatory conditions of the airway, such as LPRD.[80] Indeed, some studies reported a cellular upregulation of mucin and other genes involved in inflammatory reactions (ie, vascular endothelial growth factor, fibroblast growth factor 2, matrix metalloproteinase 1, CA III, and Sep70) after exposure to low pH (Table 3). Particularly, Johnston et al showed reduced MUC4 and MUC5AC expression in LPRD laryngeal biopsies of vocal folds.[36] Overall, if it is suspected that an acid exposure and pepsin could increase the expression of mucins,[38] the majority of experimental studies have indicated a depletion of some MUC genes related to chronic LPRD compromising epithelial restoration after chronic injury.

The third noxious impact of LPRD on vocal folds concerns the depletion of squamous epithelial heat shock protein involved in cellular protection from stress. Two studies reported a depletion of squamous epithelial heat shock protein 70 (Sep70) by acidic pepsin, suggesting an increased risk of vocal fold trauma (Table 3).

Histopathology

To date, increasing evidence has supported the development of morphologic changes in the laryngeal mucosa, especially the vibratory margin of the vocal folds (MVF), which results in a chronic inflammatory condition.[21,26,38]

The normal vocal fold epithelium corresponds to stratified squamous cells connected by apical junctional complexes that form a resistant barrier.[81] The junctional complexes ensure efficient epithelial resistance to irritative or mechanical traumas that seem impaired by LPRD. Thus, it seems that the inflammatory reaction causes dilatation of intracellular vocal epithelium space (Tables 2 and 3). Particularly, intracellular pepsin can decrease the expression of certain cell adhesion molecules, such as E-cadherin, which suggests but does not directly demonstrate a defect in the integrity of the laryngeal epithelial barrier.[21,26,36] Notwithstanding, it is not clear whether the reduction in E-cadherin expression is related to the effect of the acidic pepsin or to the inflammatory reaction.[21] Clear conclusions still remain limited because only a few studies were able to perform biopsies in the context of LPRD for ethical rationale. It is for this reason that LPRD studies based on animal models were conducted, especially on pigs and dogs because of their physiological and immunologic similarities.[82] Precisely, substantial laryngeal mucosa changes could occur after bile and trypsin exposure, such as intraepithelial inflammation, vocal fold squamous mucosal thickening and metaplasia, erosions, ulcers, stromal and periglandular infiltrations, and fibrosis, in a canine model.[18] Cohen et al supported these results with observations of significantly more cellular infiltrates in canine vocal folds exposed to pepsin than in control dogs.[19] In a rabbit model, Hu et al found an increase in the intercellular spaces of the vocal fold mucosa, which, such as in the human study of Rees et al, was also characterized by a lymphocytic infiltrate.[25,33] The mucosal thickening and cell proliferation noted in the study of Adhami et al was also observed in the rat laryngeal samples of Shimazu et al's study.[27] Moreover, another study suggested that acute pepsin exposition rapidly increases the epithelial leakage, thus decreasing the ability of the epithelial barrier to restrict movement through paracellular or transcellular pathways.[31] These observations support the potential of reflux to have easier access to the underlying lamina propria (LP) and amplify the damaging effects of the disease.[31] Finally, it has been suggested that morphologic changes related to the chronic inflammation could induce damage to the micro-ridge structure (covering the vocal fold epithelial surface and contributing to mucus adherence[83]) and negatively impact the defense of the epithelium against reflux irritations. To date, little evidence has supported this point.[34]

Clinical studies

The retrieved clinical studies suggested significant pejorative aerodynamic (ie, maximum phonation time [MPT]) and acoustic (ie, %Jitter and %Shimmer) measurements in patients with LPRD compared with healthy subjects (Tables 5), which supports the presence of objective alterations in the vibratory processes of the vocal folds. Prospectively, some studies have reported an improvement in inflammatory laryngostroboscopic findings (ie, sticky mucus, posterior commissure hypertrophy, vocal fold ulcerations, redness, edema, and granulation) after treatment (Table 6). Strangely, only a few studies examined aerodynamic measurements, such as translaryngeal airflow, subglottal pressure, MPT, and phonatory quotient, although these measures provide direct indicators of laryngeal physiology. The reported studies showed mixed results between the authors who did not find significant improvement in aerodynamic measures after medical[49,61] or surgical[62] treatment and those who found substantial enhancements.[13] The various treatment approaches and durations, as well as the epidemiological differences between studies, may largely explain these controversial results.

It is well known that subtle voice changes can be even more difficult to detect by the current perceptual assessment of the physician, especially in mild or moderate hoarseness related to reflux.[13] For this reason, many authors use acoustic measurements to study the treatment efficiency of voice quality, which provides indirect information about the vibration characteristics of patients. In LPRD, the studies reported controversial results, apparently imputed to methodological differences between studies in the measurement of the acoustic cues (Table 6).[5,63] However, a general tendency still reported significant improvement in shimmer and jitter after surgical or medical reflux treatments in most research conducting an empirical treatment with an adequate duration.[3,13,47,55,60,61] Regarding the time needed to improve laryngeal signs and symptoms,[3,84] we think that the controversial results of the studies assessing the acoustic parameters after only 4–8 weeks[49,51,57,74] are biased by the shorter period of re-

covery. Regarding the controversy related to aerodynamic results, it is possible that the variability of results found between studies depends on the epidemiological characteristics of the studies (ie, LPRD severity, treatment, selection criteria, etc). In a general manner, it seems probable that LPRD is associated with voice impairments, and the vibration alterations of the MVF are all dependent on an increase in vocal impairment in patients with severe LPRD.[64]

Pathophysiological model

During the past two decades, a few studies have investigated the pathophysiological mechanisms underlying the development of voice disorders in LPRD. Initially, some claimed that hoarseness was related to edema of the vocal folds, but according to recent studies, this claim remains controversial.[3,13] Through this systematic review of the literature, we collected meaningful information on vocal fold functioning in LPRD. The vibration of the MVF depends on several biomechanical properties, such as the vocal fold length, mass, elasticity, viscosity, and rigidity as well as the lubrication and humidification of the tissue.

Human vocal folds have very specialized and unique laminar architecture.[81] Under the squamous epithelium are three layers of connective tissue, known as the LP, with a distinct structure and different mechanical properties. The superficial layer of the lamina propria (SLLP), also called Reinke space, is located just under the epithelium. Together, the intermediate layer (rich in elastic fibers) and the deep layer (rich in collagen fibers) form the vocal ligament. Under the LP lies the thyroarytenoid muscle or the vocal muscle.[85] In the body-cover model of vibration, the cover is composed of the epithelium, and the pliable SLLP is essential to normal voice production. The cellular composition of the SLLP is very sparse and consists of fibroblasts and macrophages. Its "jelly-like" structure is due to a very loose elastic and collagen fibrous scaffolding and to the interstitium molecules of the extracellular matrix. The cover is more compliant, less rigid, and less viscous than the vocal fold body, which is a very viscous, rigid structure and not very compliant. Knowledge related to this information remains critical to understanding most of the pathophysiological mechanism underlying the development of some vocal disorders, such as LPRD, because any alteration of the biomechanical properties of the vocal folds' main vibrator, the mucosal cover, will impact the amplitude and regularity of vibration.

Vocal fold cover

In healthy subjects, a high compliance, a low rigidity, and an optimum viscosity characterize the vocal fold cover layer and mucous coat hedging the tissue, which allows movement of the MVF. Regarding the chronic downregulation of mucin genes by acidic pepsin, it seems highly probable that LPRD is associated with a chronic dehydration of mucous, leading to sticky mucus, which has been well described in some clinical studies.[3,64,84] As shown in a previous study,[86] mucous dehydration (and its increased viscosity) could have consequences on the vibration process, especially on the amplitude of the free edge of the MVF. Thus, a rise in mucus viscosity could reduce the amplitude of the mucosal wave (voice power or intensity) and shorten the closed phase during phonation without apparent changes in the vocal folds themselves.[86] In addition, dryness of the SLLP has been recently suggested as additional microscopic change related to reflux.[5] Indeed, pepsin is known to impact the transport ions and water regulating cell volume, which leads to potential modifications of the vocal fold hydration.[87] Reduced hydration of the SLLP may be associated with a reduction of the thickness of the cordal cover, potentiating the stability and the decrease of the wave amplitude, which is highlighted in some clinical studies.[64]

Moreover, the decrease in some protection mechanisms (ie, mucus coat[36,38,41] and bicarbonate production [CA III][20,21,41]) may favor the occurrence of microtrauma in the epithelium, which is illustrated in part by the depletion of junction molecules (E-cadherin) and the dilatation of paracellular spaces.[21,23,26] Microtraumas of the vocal fold could alter the resistance of the epithelium by a dehiscence of the intercellular and interlayer junctions, and the mooring of the epithelium to its basal membrane.[83] Such microtraumas could undeniably lead to benign lesions of the mucosal cover, including destructive lesions, such as sulci or nodules, which are known for considerable lesions at the level of the basal membrane.[88] These lesions can be present microscopically in some cases of LPRD. In addition, the negative loop highlighting the cough and throat clearing secondary to the subsequent accumulation of sticky mucus and globus sensations may be responsible for an exacerbation of the tension applied to the mucosa and thus increase the vocal microtraumas. Inflammatory reactions in the SLLP are common clinical observations in cases of LPRD. This includes not only diffuse redness in the vocal folds but also localized whitish, dull, and avascular zones with a drastically reduced mucosal wave (Figure 2).

As expected, some studies reported the presence of an inflammatory infiltrate in the MVF associated with the expression of inflammatory proteins in the extracellular matrix.[18,19,25,27,33] Because the compliance of the vocal cover is determined by the composition of the Reinke space,[89,90] we may postulate that (1) the presence of additional cells and liquids, (2) the destruction of some important proteins composing the matrix (decorin, elastic fibers, collagen, hyaluronic acid), and (3) the production of many inflammatory proteins modify the biomolecular composition and biomechanical properties of the superficial layer of the vocal folds. Thus, the cordal cover could be less compliant, more viscous, and rigid, thus impacting the quality of the vibratory process. The increased viscosity involves a greater difficulty to start and maintain the movement of the vocal folds, and the reduction of the rigidity implicates a greater difficulty to maintain the stability of the pitch; both of these events lead to irregularities in the vibratory process.[89] The chronic irritation and inflammatory reaction may be associated with the development of morphologic changes, such as keratosis, dysplasia, and epithelial thickening of the vocal folds.[5,18,27,91–93] Naturally, all morphologic changes in the epithelium of the MVF (ie, thickening, metaplasia, dysplasia or keratosis, vocal process ulceration, or granuloma) may increase the mass of the vocal cover, requiring a greater subglottic pressure to move the MVF (Figure 3). The direct impact of the thickening of the epithelium of the vocal

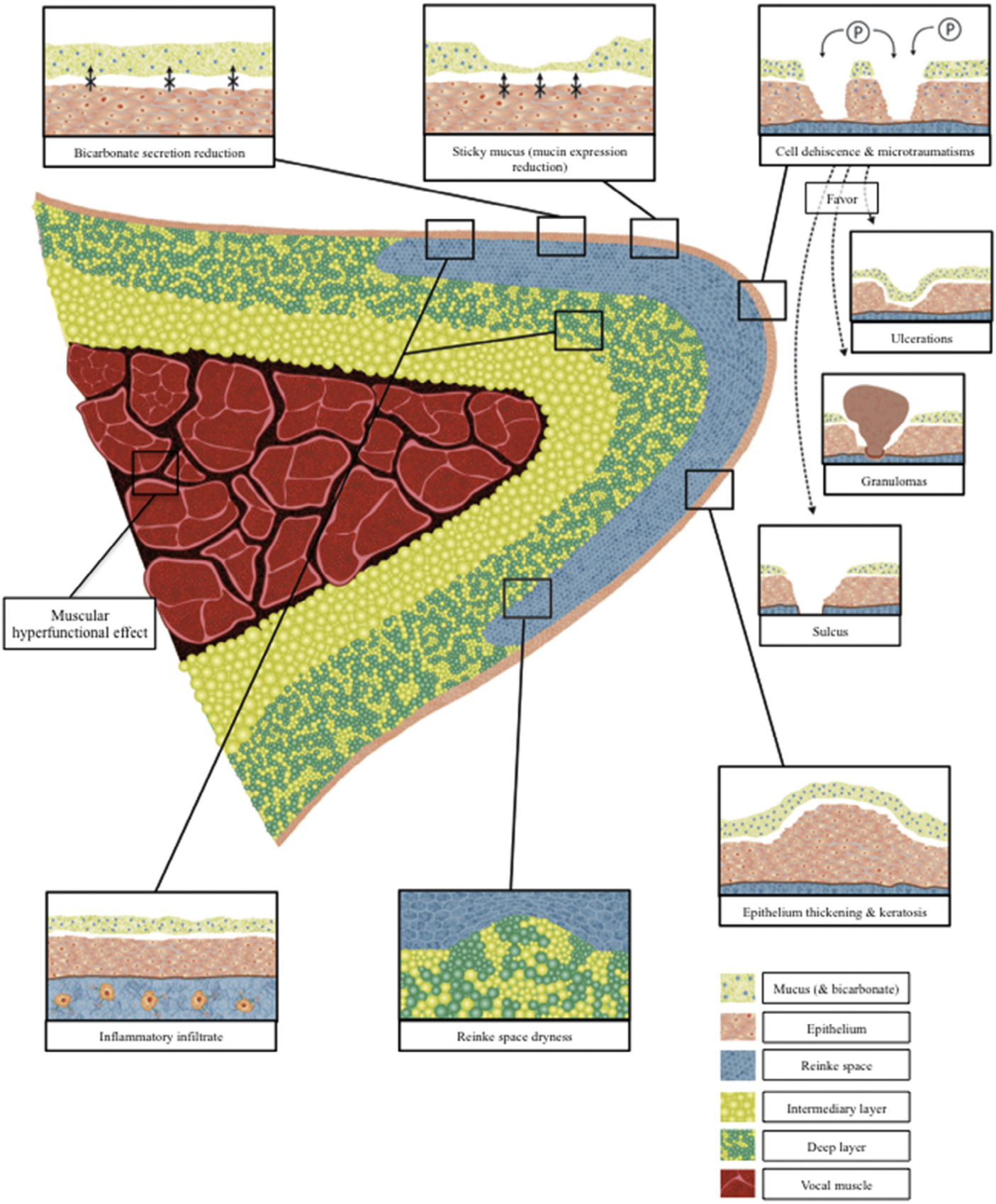

FIGURE 2. The pathophysiological mechanisms underlying the development of hoarseness related to LPRD. Hoarseness related to LPRD could be due to several mechanisms including the reduction of bicarbonate secretion, the presence of sticky mucus (related to the reduction of the mucin expression), cell dehiscence and microtraumatisms in the vibrating epithelium (which favors the occurrence of ulcerations, granulomas, and sulcus), epithelium thickening and keratosis, Reinke space dryness, inflammation infiltrate, and muscular hyperfunctional effect (compensatory behavior).

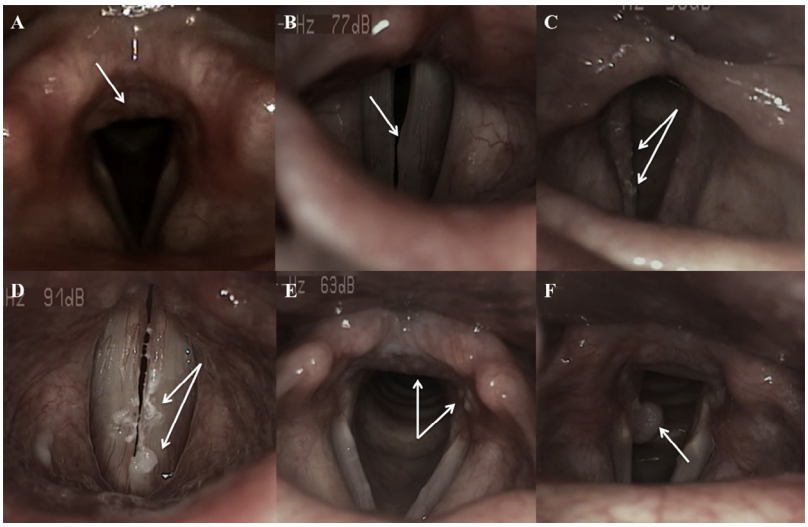

FIGURE 3. Morphologic changes of the laryngeal epithelium (videolaryngostroboscopic images). Hypertrophy of the posterior commissure (**A**), thickening or edema of the epithelium (**B**), ulcerations and microlesions (**C**), sticky mucus (**D**), keratosis (**E**), and granuloma (**F**) characterized LPRD findings and may alter the vocal folds' biomechanical characteristics.

folds on both compliance and resistance properties of the tissue remains unknown to date.

Intermediary layer and cordal body

Currently, we have not found evidence supporting an involvement of the intermediary layer in the pathophysiology of LPRD, because most of the research has focused on the effects of the inflammatory reaction on the vocal fold cover. In contrast, concerning the cordal body, the thyroarytenoid muscle could be involved in hyperfunctional behaviors (forcing) that are described in several LPRD studies.[16,42,43,94] When phonation is produced in the context of increased constriction of both vocal folds and surrounding regions (ventricle bands), airflow through the membranous glottis is reduced, and subglottal pressure is increased. These phenomena are characterized by a reduction in MPT and acoustic measurement perturbations (shimmer).[95] Moreover, as proposed in a recent paper, the possible muscular hyperfunctional effect may also be secondary to a surface inflammatory reaction.[5] Other minor pathophysiological mechanisms may be involved in voice changes, such as the edema of the supraglottic structures (resonating cavities) changing the voice tone. Finally, it is important to emphasize that we are not all genetically equal in our vocal fold tissue responses to gastric reflux.

This model is based on current literature that has some limitations. The diagnosis and treatment of reflux remain controversial, and the differences between studies may induce comparison bias. Moreover, the methodological approaches used to assess objective findings (ie, not blinded laryngostroboscopic evaluations, different approaches to measure acoustic parameters, etc) may also limit the elaboration of clear conclusions. In addition, LPRD is a chronic condition that occurs many times per day over many months in human patients.[96] Hence, the development of certain laryngeal lesions and morphologic changes may take time in humans and are very difficult to replicate in animal models. Animal models have limitations regarding the different duration and frequency needed to develop chronic conditions and their anatomical and histologic differences (topographic localization of the larynx; biomechanical composition of the vocal folds; biomolecular differences of pepsin, trypsin, and other molecules involved in the disease; types of reflux [gaseous *vs.* liquid] etc).

IMPLICATIONS FOR PRACTICE

To date, there is no multifactorial model proposing a pathophysiological hypothesis of the occurrence of hoarseness in LPRD. In this paper, based on the current literature, we propose that voice impairment may be related to a myriad of macroscopic and microscopic mechanisms not always objectified with conventional approaches (videolaryngostroboscopy or high-speed camera), especially in mild or moderate LPRD. These data suggest a complex physiology in response to mucosal acid and pepsin exposure. The suspicion and the identification of the mechanisms underlying the development of hoarseness related to reflux could help guide the further trials studying voice quality in LPRD. Moreover, the better knowledge of the pathophysiological mechanisms and the prevention of these mechanisms remain crucial for the voice professionals (ie, teachers, singers, etc) who are more subject to chronic hoarseness and the development of benign lesions. Regarding the findings exposed in this paper, it seems important to develop new laryngoscopic approaches to objectify the microscopic findings involved in the development of LPRD in human subjects. Further studies are needed to elucidate the biomolecular mechanisms underlying the development of mucosal morphologic changes and the impact of the LPRD lesions in the development of vocal organic lesions.

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