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Association Between Laryngopharyngeal Reflux and Benign Vocal Folds Lesions: A Systematic Review.

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Objectives: To investigate the role of laryngopharyngeal reflux (LPR) in the development of benign lesions of the vocal folds (BLVF).

Methods: PubMed, Cochrane Library, and Scopus were searched by three independent investigators for articles published between January 1990 and November 2018 providing substantial information about the role of LPR in the development of nodules, polyps, cysts, Reinke's edema, and sulcus vocalis. Inclusion, exclusion, diagnostic criteria and clinical outcome evaluation of included studies were analyzed using Preferred Reporting Items for Systematic Reviews and Meta-Analyses criteria.

Results: Of the 155 relevant publications, 42 studies were included. Thirty-five were clinical studies and seven were experimental research studying the impact of reflux on vocal fold tissue. Only seven clinical studies utilized objective LPR diagnoses (pH monitoring), suggesting an association between LPR and the development of nodules, polyps, and Reinke's edema. These studies were characterized by a substantial heterogeneity due to discrepancies in inclusion/exclusion criteria, diagnostic methods, and clinical outcome evaluation. The few basic science studies on this topic support that LPR creates an environment that may predispose to BLVF through changes in defense mechanisms of the vocal folds, cell-to-cell dehiscence, inflammatory reaction of the vocal folds, and reaction to phonotrauma.

Conclusions: Caustic mucosal injury from LPR could cause increased susceptibility of the vocal fold mucosa to injury and subsequent formation of nodules, polyps, or Reinke's edema. However, the heterogeneity and the low number of high-quality studies limit the ability to draw definitive conclusions. Future clinical and experimental studies are needed to better identify the role of reflux in development of BLVF.

Key Words: Reflux, laryngopharyngeal, benign, lesion, vocal fold, vocal cord, nodules, polyps, cysts, Reinke's edema, sulcus.

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INTRODUCTION

Laryngopharyngeal reflux (LPR) is an inflammatory condition of the upper aerodigestive tract tissues related to direct and indirect effect of gastric or duodenal content reflux, which induces morphological changes in the upper aerodigestive tract.[1] LPR-related symptoms are found in approximately 4% to 10% of outpatients visiting otolaryngology–head and neck surgery departments[2] and up to 50% of patients in voice centers.[3] It has long been suggested that LPR could play a key role in the development of benign lesions of the vocal folds (BLVF)[4] such as nodules, polyps, Reinke's edema, sulcus vocalis, and cysts.[5–8] However, the pathophysiological mechanisms through which LPR predisposes to BLVF and the epidemiological evidence linking LPR and BLVF still remain unclear. BLVFs are one of the three most prevalent conditions associated with dysphonia and involve a significant cost ranging from US$577 to US$953 per patient per year.[9,10] According to a recent pathophysiological model explaining the development of hoarseness related to LPR, vocal fold mucosa could be more vulnerable to mechanical and biochemical stresses of normal and abnormal phonation processes in the setting of reflux.[11]

However, this hypothesis is still theoretical, and to date, no systematic review has been conducted to evaluate the different clinical and basic science studies linking LPR to the development of BLVF. The aim of this article was to

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review the current literature about the role of LPR in the development of BLVF, especially nodules, polyps, cysts, Reinke's edema, and sulcus vocalis. Following review of appropriate experimental and clinical studies, we propose a new integrative model of pathophysiological mechanisms underlying the development of benign lesions in the context of LPR.

MATERIALS AND METHODS

The criteria for considering studies for the systematic review were based on the population, intervention, comparison, and outcome framework.[12]

Types of Studies

Clinical trials, both prospective and retrospective studies, and basic science experimental research published in peer-reviewed journals were included in this review. Studies were included if they explored the impact of reflux on the mucosa of the human vocal folds relative to BLVF. We included studies published in English, Spanish, and French.

Participants and Inclusion/Exclusion Criteria

Articles were included for analysis if they clearly described means of LPR diagnosis and methods for BLVF diagnosis. Relative to LPR diagnosis, articles were included if they attempted rigorous diagnosis of LPR through symptoms, exam findings, or objective testing. Patients with positive pH-metry or multichannel intraluminal pH-impedance monitoring (MII-pH) were considered as LPR patients in this analysis; those with a clinical diagnosis based on symptoms or exam findings alone were considered as suspected LPR patients. Included studies established diagnosis of benign vocal fold lesions through videolaryngostroboscopy or histological examination after phonosurgery. To be included, a study had to clearly explain their taxonomy of any included benign lesions such as nodules, polyps, cysts, fibrous masses, pseudocysts, and sulcus vocalis.[13]

Outcomes

The first study outcome was review of potential causal association between LPR and BLVF through clinical studies. The second study outcome was review of basic science studies to evaluate ways in which modification of microscopic and macroscopic properties of vocal fold mucosa by LPR might lead to the development of BLVF. Heterogeneity among included articles in the patient population, means of LPR diagnosis, and outcomes measures limited the ability to combine data statistically into a formal meta-analysis, limiting analysis of the current systematic review to qualitative rather than quantitative summary of the available information.

Intervention and Comparison

Because the aim of the study was to analyze the potential relationship between both clinical conditions rather than assess the impact of LPR treatment on BLVF, included studies did not need to detail treatment approaches or response.

Search Strategy

Three authors (J.R.L., M.R.B., C.F.) conducted a PubMed, Cochrane, and Scopus search to identify articles published between January 1990 and September 2018 concerning the role of LPR in the development of BLVF (i.e., nodules, polyps, cysts, Reinke's edema, and sulcus vocalis). Clinical and experimental studies were screened if they had database abstracts, available full texts, or titles referring to the condition. The following keywords were used: "reflux," "laryngitis," "laryngopharyngeal," "gastroesophageal," "benign," "lesion," "vocal," "nodule," "cyst," "polyp," "edema," and "sulcus." Final article selection was determined by these three authors, who provided a critical analysis of the publication's content and summarized the data of the selected articles. The review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist for systematic reviews.[14] Institutional review board approval was not required.

Epidemiological Characteristics, Reflux Diagnostics, and Outcomes

The investigators analyzed trials for number of subjects, study design, inclusion and exclusion criteria, quality of trial, evidence level, symptoms, and signs used for LPR diagnosis. Previous studies have highlighted the ways in which other conditions such as allergy, dryness, and phonotrauma might mimic LPR, and included studies emphasizing approaches to LPR diagnosis, which helped to limit potential impact of confounding issues.[15] Methods used for LPR diagnosis were carefully analyzed, and risk of bias was assessed using the Tool to Assess Risk of Bias in Cohort Studies developed by the Clarity Group and Evidence Partners.[16]

RESULTS

Characteristics of Studies

Initial screening identified 121 clinical articles; some of these articles focused on LPR and several categories of BLVF, whereas some focused on the intersection between LPR and a single category of BLVF. Counting articles across several categories when appropriate, 32 of these articles are related to nodules, 29 to polyps, 18 to cysts, 32 to Reinke's edema, and 10 to sulcus vocalis. When filtered through the inclusion and exclusion criteria, a total of 35 articles were kept for analysis: 16 are related to nodules,[5,7,17–30] eight to polyps,[7,20,22–24,26,31,32] three to cysts,[18,20,30] five to Reinke's edema,[22,24,26,33,34] and three to sulcus vocalis[7,18,35] (Fig. 1).

Of these 35 articles, the majority of clinical studies had low levels of evidence and were characterized by important heterogeneity in diagnostic methods, exclusion and inclusion criteria, and clinical outcomes (Table I). LPR diagnosis was performed with pH monitoring in seven studies[5,22,24,26,29,33,34]; among these, the criteria of positive pH monitoring test differed substantially from one article to the next, and none utilized impedance testing. The 28 remaining studies based LPR diagnosis on symptoms ± findings, with a few authors using validated clinical tools.[18,21,32] LPR laryngoscopic findings were not taken into consideration for diagnosis in eight studies,[5,19,20,22,23,27,30,31] and only one author assessed exam findings in a blinded fashion[33] (Table I). Two authors utilized gastroesophageal reflux disease (GERD) criteria for LPR diagnosis.[7,28] As for exclusion criteria, only three authors excluded confounding conditions that may lead to misdiagnosis of reflux.[7,21,23] Some authors included smokers (N = 5),[18,22,30,31,33] patients with excess alcohol consumption (N = 3),[18,30,33] or patients with chronic rhinosinusitis (N = 2).[7,31] No study excluded patients with allergies. The literature that attempts to link LPR with BLVF widely includes

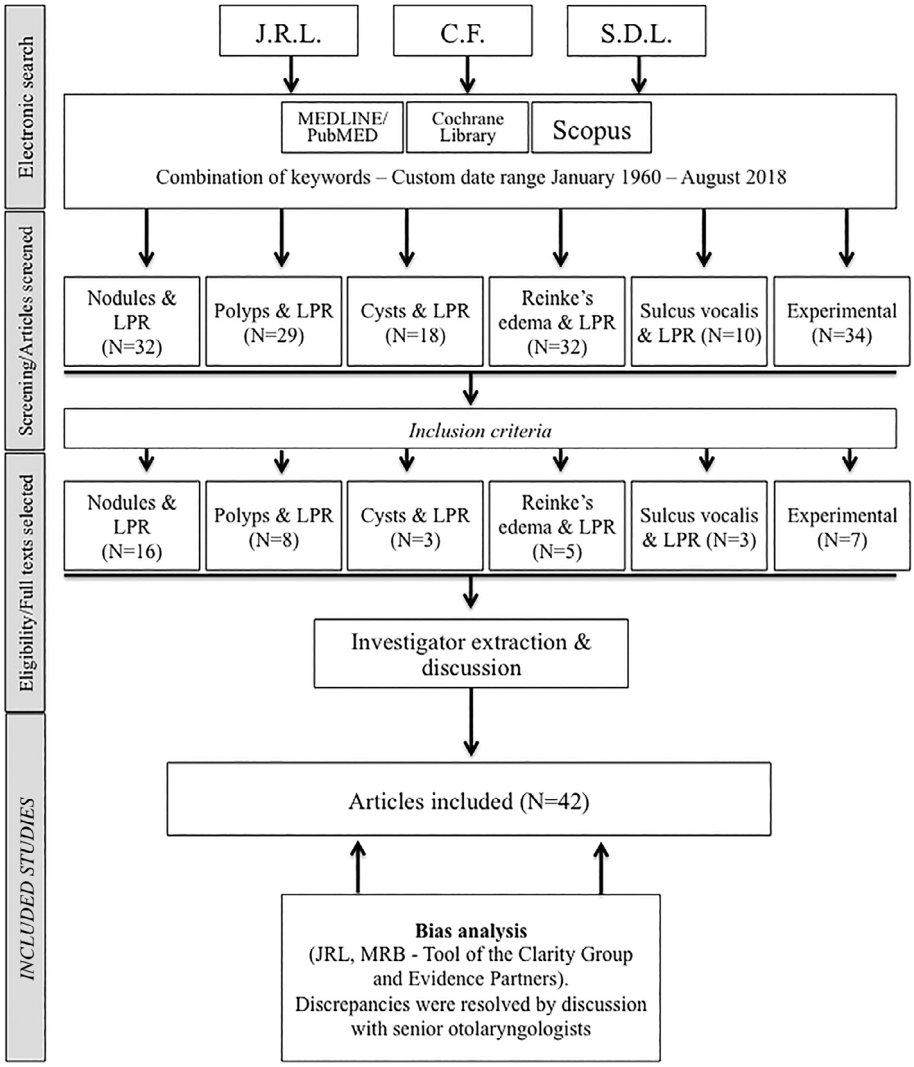

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart shows the process of article selection for this study. C.F. = author Camille Finck; J.L.R. = author Jerome R. Lechien; LPR = laryngopharyngeal reflux; MRB = author Maria Rosaria Barillari. S.D.L = author Serge D. Le Bon.

potential confounders that make it difficult to isolate LPR as an independent risk factor for vocal fold lesions.

Additionally, there were 34 basic science articles identified and seven selected for ultimate inclusion in the qualitative analysis.[36–42] As compared to clinical articles that focused on a patient population, these studies examined the relationship between LPR and phonotraumatic lesions in the laboratory setting.

Laryngopharyngeal Reflux and Nodules

The literature search identified 32 relevant publications; among these, 16 met our inclusion criteria (Table II). Four and five studies have, respectively, suggested the coexistence of suspected LPR and nodules in patients with nonspecific laryngopharyngeal symptoms[21,23,25,27] and in voice professionals (i.e., singers and teachers)[7,18,25,28,30] without exhibiting reliable LPR diagnostic methods. One study stated that nodules and LPR-related symptoms were more prevalent in elderly patients than younger subjects.[19] Recently, Tasli et al. evaluated pepsin levels in vocal fold nodule specimens that were removed in patients with long-term history of dysphonia; using Western blot and enzyme-linked immunosorbent assay analyses, these authors did not identify pepsin in operated nodule specimens.[17] Another article reported that patients with LPR symptoms have poorer response to vocal fold steroid injection for nodule treatment.[20] As shown in our bias analysis, none of the studies in this paragraph used pH monitoring to confirm LPR diagnosis (Table I).

Five studies investigated the relationship between LPR and nodules using an objective approach to LPR diagnosis.[5,22,24,26,29] In 1998 and 1999, respectively, Kuhn et al. and Ulualp et al. reported a significantly higher prevalence of pharyngeal acid reflux events in patients with nodules in comparison with healthy subjects.[5,29] More specifically, Ulualp et al. identified LPR in 78% of patients with nodules, whereas healthy subjects had positive pH monitoring in only

TABLE I.
The Epidemiological Characteristics of Studies Investigating the Relationship Between Reflux and Benign Lesions of the Vocal Folds.

References	Design	LPR Diagnostic	Exclusion Criteria	Symptoms/Findings Definition	Reliable Finding Assessment	Blinded Assessment
Van Houtte, 2010[25]	Pros uncontr	Probably no	No	Yes	Probably no	No
de Bortoli, 2012[21]	Pros uncontr	Probably yes	Probably yes	Yes	Yes	No
Arruda Henry, 2011[23]	Pros uncontr	Probably no	Probably yes	Probably no	No	No
Catalano, 2004[27]	Pros contro	Probably no	Probably no	Probably no	No	No
Lundy, 1999[30]	Pros uncontr	Probably no	No	Probably no	No	No
Nacci, 2019[18]	Pros contro	Probably yes	No	Yes	Yes	No
Pereira, 2015[7]	Pros contro	Probably no	Probably yes	Probably no	Probably no	No
Pérez Fernández, 2003[28]	Pros contro	No	No	Probably no	Probably no	No
Çiyiltepe, 2017[19]	Pros uncontr	No	No	No	No	No
Tasli, 2018[17]	Pros contro	Probably no	No	Probably yes	Probably no	No
Wang, 2015[20]	Pros uncontr	Probably no	Probably no	Probably yes	No	No
Kuhn, 1998[5]	Pros contro	Yes	No	Probably no	No	No
Beltis, 2011[22]	Pros contro	Yes	Probably no	Probably no	No	No
Chung, 2009[24]	Pros contro	Yes	Probably no	Yes	Yes	No
Kantas, 2009[26]	Pros contro	Yes	Probably no	Yes	Yes	No
Ulualp, 1999[29]	Pros contro	Yes	No	Yes	Yes	No
Akdogan, 2015[31]	Pros contro	No	No	No	No	No
Siupsinkiene, 2013[32]	Pros contro	Probably yes	Probably no	Yes	Yes	No
Kamargiannis, 2011[34]	Pros contro	Yes	Probably no	Yes	Yes	No
Katsinelos, 2009[33]	Pros uncontr	Yes	No	Probably no	Yes	Yes
Myint, 2016[35]	Retro uncontr	Probably no	No	Probably yes	Yes	No

Epidemiological analysis was performed using the Tool to Assess Risk of Bias in Cohort Studies.[16]

For the diagnostic of reflux, the following criteria were used for the bias assessment: No = not defined; Probably no = diagnostic based on suspected symptoms ± signs; Probably yes = diagnostic based on symptoms and signs according to clinical validated tools.

Exclusion criteria adopted in studies were analyzed, and we considered the exclusion of the following seven categories (cat.) of confounding conditions as optimal: cat. 1: patients with ENT and respiratory toxic or infectious disorder(s) within the last month; cat. 2: smoker, alcoholic, and subjects with active allergy; cat. 3: patients with antireflux treatment already started in the previous month; cat. 4: patients with current/history of malignancies, radiotherapy, laryngeal trauma, and head and neck previous surgery; cat. 5: patients with other laryngeal lesions such as pseudocyst, leukoplakia, papillomatosis; cat. 6: patients with severe neurologic and psychiatric disorders; and cat. 7: subjects with other ENT diseases that may lead to confounding ENT complaints. No = if there is no or one category of exclusion criteria reported in the study; Probably no = if one to three categories of exclusion criteria were applied; Probably yes = if three to five categories of exclusion criteria were applied; Yes = if six or more categories of exclusion criteria were applied in the study.

Concerning the symptoms/findings definition, the following criteria were used for the bias analysis: No = symptoms and signs were not defined; Probably no = symptoms or signs were partially defined; Probably yes = symptoms or signs were clearly defined; Yes = symptoms and signs were clearly defined.

Regarding the finding assessment, the following criteria were considered: No = there was no specified method for the assessment; Probably no = the assessment was subjective without tool; Probably yes = assessment was performed with unvalidated tool; Yes = assessment was made with validated tool.

contro = controlled studies; ENT = ear, nose, and throat; LPR = laryngopharyngeal reflux; Pros = prospective studies; Retro = retrospective studies; uncontr = uncontrolled studies.

21% of cases.[29] In the study of Kuhn et al., LPR was identified in 64% of nodule patients and 18% of control subjects.[5] This relationship between LPR and nodules has been supported by Beltis et al., who assessed the prevalence of LPR in patients with nodules compared to patients suffering from GERD.[22] According to pH monitoring findings that differentiated distal reflux alone (GERD) from those who had proximal reflux (LPR), these authors reported a nodule rate of 60% in patients with LPR, double the rate of nodules in the GERD group. In contrast, Chung et al. did not identify a significantly higher prevalence of LPR (pH-metry), pathological Reflux Finding Score (RFS), or Reflux Symptom Index (RSI) in patients with nodules compared to subjects who complained of LPR symptoms without BLVF.[24] The role of LPR in both the healing and the recurrence of BLVF was examined in one study[26]; its authors observed that the persistence of LPR after surgery negatively influenced the epithelization of vocal folds in patients who had surgery for polyps and Reinke's edema, but not those undergoing phonosurgery for nodules.

Laryngopharyngeal Reflux and Polyps

Twenty-nine studies looked at the co-occurrence of LPR and polyps in dysphonic patients, but only eight met our inclusion criteria (Fig. 1, Table III). The epidemiological characteristics of included studies are described in Table I. Five publications studied the association between LPR and polyps without objective LPR diagnosis. These studies exhibited a low level of evidence in the demonstration of an association between LPR and polyps.[7,20,23,31,32] However, similar to nodules, it was reported that LPR negatively

TABLE II.
The Characteristics of Studies Investigating the Relationship Between Reflux and Nodules.

References	Design	EL	LPR Diagnostic	Samples/Patients Characteristics	Outcomes	Results	Main findings
Tasli, 2018[17]	Prospective, controlled	IIIb	Symptoms and signs	Nodule specimens (N = 28), CT (N = NP)	Nodule pepsin level (WB, ELISA)	No difference between groups	There is no pepsin identified in nodule specimen.
Nacci, 2019[18]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: Healthy non-singer (N = 60)	Clinical LPR		In singers, authors demonstrated a significant association between suspected LPR and nodules.
				Gr 2: Singing students (N = 56)	RSI and RFS	Higher in Gr 2	
					VLS: Nodules, cysts, sulcus	Higher in Gr 2	
Çiyiltepe, 2017[19]	Prospective, uncontrolled	IV	Symptoms and signs	Elderly patients with dysphonia (N = 91)	Patients with LPR (prevalence)	N = 10	Suspected LPR and VF nodules are usually found in elderly patients with dysphonia. An indirect association may be suggested.
					Patients with nodules (prevalence)	N = 22	
Wang, 2015[20]	Prospective, uncontrolled	IV	Symptoms and signs	Nodules (N = 49)	Improvement of perceptual VQ		LPR symptoms are associated with poor response to VF steroid injection in patients with nodules. Patients without suspected LPR have better response than LPR patients.
				Polyps (N = 47)	Grade, roughness, breathiness	non-LPR > LPR	
				Retention cysts (N = 30)			
Pereira, 2015[7]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: teachers (N = 90)	GERD and LPR symptoms	Higher in Gr 1	LPR symptoms are associated with the development of nodules in teacher population.
				Gr 2: healthy nonteacher (N = 90)	Association between LPR and nodules	Higher in Gr 1	
de Bortoli, 2012[21]	Prospective, uncontrolled	IV	Symptoms and signs	Suspected LPR patients (N = 41)	Association between LPR and nodules	Positive association	Nodules are usually found in patients with LPR symptoms and signs (suspected LPR).
Beltsis, 2011[22]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: LPR (N = 68)	LPR episodes (pH monitoring)	Higher in Gr 1	LPR is more prevalent in patients with benign lesions of the VF compared with typical GERD patients
			RFS >7	Nodules (N = 20)	Patients with nodules (LPR/GERD)(prevalence)	60%–30%	
			pH monitoring	Gr 2: GERD (N = 24)			
Arruda Henry, 2011[23]	Prospective, uncontrolled	IV	Symptoms and signs	Gr 1: GERD and dysphonia	Prevalence of nodules	Higher in Gr 1	Nodules are associated with dysphonia in GERD patients.
				Gr 2: GERD without dysphonia	Prevalence of polyps	Higher in Gr1	
Chung, 2009[24]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: patients with BLVF	LPR prevalence (pH monitoring)	No difference between groups	LPR is not more prevalent in patients with nodules in comparison with patients with symptoms of LPR.
			pH monitoring	Gr 1B: nodules			
				Gr 2: LPR symptoms (N = 200)			
Van Houtte, 2010[25]	Prospective, uncontrolled	IV	Symptoms and signs	Patients with dysphonia (N = 882)	Nodules (prevalence)	15% of patients	Nodules and suspected LPR are commonly associated with dysphonia, especially in voice professional users. Both conditions can be associated.
					LPR (prevalence)	9% of patients	
					Nodules and LPR (prevalence)	Higher in voice professionals	
Kantas, 2009[26]	Prospective, controlled	IIIb	Symptoms and signs	LPR patients with phonosurgery	Postoperative RSI	Higher in Gr 1	LPR does not seem to influence epithelization and recurrence nodules after partial or total decortication.
			pH monitoring	Reinke's edema (N = 40)	Postoperative RFS	Higher in Gr 1	

TABLE II.
(*Continued*)

References	Design	EL	LPR Diagnostic	Samples/Patients Characteristics	Outcomes	Results	Main findings
				Nodules or polyps (N = 72)	Epithelialization and recurrence of	No difference between groups	
				Gr 1: PPIs postsurgery	Nodules		
Gr 2: no PPIs postsurgery							
Catalano, 2004[27]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: suspected LPR patients (N = 110)	Nodules (prevalence)	Higher in Gr 1	Patients with nodules have a significantly higher prevalence of esophagitis compared to healthy subjects.
				Gr 2: healthy subjects (N = 117)			
Pérez Fernández, 2003[28]	Prospective, controlled	IIIb	NP	Gr 1: teachers with dysphonia (N = 120)	Nodules (prevalence)	Higher in Gr 1	GERD is one of the most relevant personal factors in the development of vocal pathology.
				Gr 2: healthy teachers (N = 120)	GERD (prevalence)	Higher in Gr 2	
Ulualp, 1999[29]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: LPR with nodules (N = 9)	LPR in patients with nodules (prevalence)	78% of patients	According to pH monitoring study, LPR is more prevalent in patients with nodules compared to healthy subjects.
			pH monitoring	Gr 2: CT (N = 34)	LPR in healthy subjects (prevalence)	21% of patients	
Lundy, 1999[30]	Prospective, uncontrolled	IV	Symptoms and signs	Asymptomatic singing students (N = 65)	Prevalence of LPR findings in students with both nodules and cysts	High prevalence	In patients with nodules of the VF, LPR findings such as laryngeal erythema are prevalent.
				Asymptomatic student with nodules (N = 2) and cysts (N = 3)			
Kuhn, 1998[5]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: patients with nodules (N = 11)	LPR acid reflux episodes	Gr 1 = 7/11, Gr 1 = 2/11, higher in Gr 1	Prevalence of pharyngeal acid reflux events is significantly higher in patients with VF nodules compared with healthy CT.
			pH monitoring	Gr 2: healthy subjects			

BLVF = benign lesions of the vocal folds; CT = controls; EL = evidence level; Gr = group; ELISA = enzyme-linked immunosorbent assay; GERD = gastroesophageal reflux disease; LPR = laryngopharyngeal reflux; N = number of patients; NP = not provided; PPIs = proton pump inhibitors; RFS = Reflux Finding Score; RSI = Reflux Symptoms Index; VF = vocal fold; VLS = videolaryngostroboscopy; VQ = voice quality; WB = Western blot.

TABLE III.
Characteristics of Studies Investigating the Relationship Between Reflux and Polyps.

References	Design	EL	LPR Diagnosis	Samples/Patients Characteristics	Outcomes	Results	Main Findings
Wang, 2015[20]	Prospective, uncontrolled	IV	Symptoms and signs	Nodules (N = 49) Polyps (N = 47) Retention cysts (N = 30)	Improvement of perceptual VQ Grade, roughness, breathiness	Better for non-LPR than LPR patients	LPR symptoms are associated with poor response to VF steroid injection in patients with polyps. Patients without suspected LPR have better response than LPR patients.
Pereira, 2015[7]	Prospective, controlled	IIIb	Symptoms and signs	Gr 1: teachers (N = 90) Gr 2: healthy nonteacher (N = 90)	GERD and LPR symptoms Association between LPR and polyps	Higher in Gr 1 Higher in Gr 1	LPR symptoms are associated with the development of polyps in control population.
Beltsis, 2011[22]	Prospective, controlled	IIIb	Symptoms and signs RFS >7 GERD = 37%	Gr 1: LPR Gr 2: GERD (N = 24)	LPR episodes (pH monitoring) Prevalence of LPR and GERD in patients with polyps	Higher in Gr 1 LPR = 75%,	LPR is more prevalent in patients with benign lesions of the VF compared with typical GERD patients
pH monitoring	Polyps (N = 32)						
Arruda Henry, 2011[23]	Prospective, uncontrolled	IV	Symptoms and signs	Gr 1: GERD and dysphonia Gr 2: GERD without dysphonia	Presence of nodules in GERD patients Presence of polyps in GERD patients	Yes Yes	Nodules and polyps are associated with dysphonia in GERD patients.
Kantas, 2009[26]	Prospective, controlled	IIIb	Symptoms and signs pH monitoring	LPR patients with phonosurgery Reinke's edema (N = 40) Nodules or polyps (N = 72) Gr 1: PPIs postsurgery Gr 2: no PPIs postsurgery	Postoperative RSI Postoperative RFS Epithelialization and recurrence of Reinke's edema and polyps	Higher in Gr 1 Higher in Gr 1 Higher in Gr 1	LPR influences epithelization and recurrence of laryngeal polyps in vocal cords, after partial or total decortication.
Akdogan, 2015[31]	Prospective, controlled	IIIb	NP	Patients with polyps (N = 32) Gr 1: high level of stromal ADAM-33 Gr 2: low level of stromal ADAM-33 Gr 3: normal VF tissues (N = 36)	Suspected LPR	No difference between groups	Suspected LPR is not associated with overexpression of a disintegrin and metalloproteinase protein 33 in patients with VF polyps.
Siupsinskiene, 2013[32]	Prospective, controlled	IIIb	RSI >13 and RFS >7	Patients who had VF surgery (N = 78) Gr 1: polyps (N = 32) Gr 2: chronic laryngitis (N = 22) Gr 3: CT, normal VF tissue (N = 11)	*Helicobacter pylori* infection	Higher in Gr 2 No difference between groups	*Helicobacter pylori*, which is associated with suspected LPR, can colonize in the larynx of patients with VF polyps.
Chung, 2009[24]	Prospective, controlled	IIIb	Symptoms and signs pH monitoring	Gr 1: patients with VF benign lesions Gr 1C: polyps Gr 2: LPR symptoms (N = 200)	LPR prevalence (pH monitoring)	Higher in Gr 1C	LPR is more prevalent in patients with polyps than in patients with symptoms of LPR. LPR might play a role as an etiologic factor in polyps.

ADAM = a disintegrin and metalloproteinase protein; CT = controls; EL = evidence level; GERD = gastroesophageal reflux disease; Gr = group; LPR = laryngopharyngeal reflux; NP = not provided; PPIs = proton pump inhibitors; RFS = Reflux Finding Score; RSI = Reflux Symptoms Index; VF = vocal folds; VQ = voice quality.

influences voice outcomes of patients after surgery for polyps, as those patients without LPR had better perceptual voice quality outcomes after surgery than those with LPR.[20]

Among the authors who diagnosed reflux with pH monitoring, Chung et al. identified significant reflux events in 75% of patients with polyps.[24] The same rate of LPR has been found in patients with polyps in the study of Betis et al., whereas only 37% of healthy controls had positive pH monitoring.[22] However, the pH-monitoring diagnostic criteria used in these two studies are quite different. Finally, LPR seems to be associated with impaired re-epithelization of vocal folds in patients who had phonosurgery for polyps.[26]

Laryngopharyngeal Reflux and Reinke's Edema

Only five publications evaluated the association between LPR and Reinke's edema (Fig. 1, Table IV); however, not all of them utilized pH monitoring. In 2009, Chung et al. identified a higher prevalence of LPR episodes in patients with Reinke's edema in comparison to patients with LPR symptoms without Reinke's edema.[24] In the study of Beltsis et al., 67% of patients with Reinke's edema had positive LPR as per pH monitoring.[22] In a large cohort of patients who had gastrointestinal endoscopy, Katsinelos et al. investigated the occurrence of both chronic laryngitis and Reinke's edema. Similar to other studies, these authors found a significant association between GERD, chronic laryngitis, and Reinke's edema.[33] More recently, Kamargiannis et al. compared RSI, RFS, and histological findings of chronic laryngitis in patients with Reinke's edema and patients with both Reinke's edema and demonstrated LPR, and after carefully excluding smokers to reduce the risk of confounding bias, this group reported that LPR patients had more inflammatory processes in the laryngeal mucosa than those without reflux.[34] Finally, as found in polyps, LPR is associated with impaired re-epithelization of the vocal folds after surgical procedures for Reinke's edema.[26]

Laryngopharyngeal Reflux, Cysts, and Sulcus Vocalis

Four included studies evaluated the relationship between LPR and vocal fold cysts or sulcus vocalis; two studies focused on both cysts and sulcus, one on sulcus only, and one on cysts only as the BLVF of interest (Fig. 1). Nacci et al. exhibited a higher prevalence of both cyst and sulcus in singing students who had higher RSI and RFS scores in comparison with nonsinging students.[18] Myint et al. showed that opera students with suspected LPR had a higher prevalence of sulcus vocalis than students without LPR symptoms.[35] Pérez Fernandez and Preciado López also observed a higher prevalence of cysts and LPR in teachers without exploration of a potential link between both clinical entities.[28] The authors of these two studies suggested a possible association with LPR, but reflux was not demonstrated with pH monitoring.[18,28] Similar to nodules and polyps, Wang et al. suggested that patients who benefited from phonosurgery for vocal fold retention cysts had better postoperative perceptual voice-quality improvement if they did not have LPR symptoms.[20] Overall, no high–level of evidence research was conducted to study the relationship among LPR, cysts, and sulcus vocalis. Moreover, in the majority of these studies, there was no additional information about the type of cysts that were included (i.e., mucous vs. epidermoid cysts).

Experimental Studies

Thirty-four publications were identified, and seven met our criteria (Table V).[36–42] The impact of LPR on the mucosa of the vocal folds has mainly been studied throughout the study of pepsin. Pepsin can lead to significant impairments in cellular physiology irrespective of the pH of the reflux episodes (acid, nonacid). First, it has been demonstrated that pepsin significantly reduces some protective mechanisms of the mucosa of the vocal folds including dysregulations of the expression of carbonic anhydrase III and mucin genes 4 and 5 AC (*MUC4* and *MUC5AC*),[36–39] both leading to acidification and dehydration of the protective mucus of the vocal folds.[37] Second, experimental studies showed that pepsin downregulates the expression of E-cadherin,[37,39,40] leading to a weakness of the cell-to-cell adhesion structure favoring the occurrence of microtraumas. Microscopically, some data strongly support the presence of microtraumas in the mucosa of the vocal folds exposed to pepsin.[11,39] Third, cellular injury related to pepsin contributes to the development of inflammation, which is associated with modifications of the biomolecular composition and biomechanical properties of vocal fold tissue.[11] A recent study suggests that cellular injuries related to pepsin could be mediated by intracellular mitochondrial damage caused by internalization of pepsin into the intracellular space where the pH allows for increased pepsin activity.[43] Overall, pepsin activity in the vocal fold mucosa may lead to the reduction of some protective mechanisms, the occurrence of microtraumas, and the modification of biomechanical properties.

DISCUSSION

For a long time, otolaryngologists have treated reflux in their patients with voice complaints, and reflux treatment after phonosurgery for BLVF is commonly recommended. However, there are a small number of studies that support the association between LPR and BLVF,[5,22,24,29,33,34] and a low level of evidence characterizes most of them. Moreover, there is a large degree of heterogeneity between studies regarding inclusion, exclusion, and diagnostic criteria for LPR; this heterogeneity limits an ability to draw clear conclusions across the studies. Establishing LPR diagnosis on the basis of clinical evaluations without additional objective examination is a problem, because LPR symptoms and signs are nonspecific[1,44,45] and can usually be found in a myriad of BLVFs irrespective to reflux.[13,46]

To improve the quality of the literature associating LPR with BLVF, excluding or controlling for confounding conditions would be very helpful. However, most of the included studies do not account for possible confounders, biasing potential LPR diagnosis and clouding any relationship between LPR and benign lesions. Smoking, alcohol abuse, chronic rhinosinusitis, and allergy may be associated with laryngopharyngitis findings and misdiagnosis of reflux.[1,15,47,48]

Other potential bias includes lack of blinded analysis, and among the included studies that studied the association between BLVF and reflux through laryngoscopy exam findings,

TABLE IV.
Characteristics of Studies Investigating the Relationship Between Reflux and Reinke's Edema.

References	Design	EL	LPR Diagnostic	Samples/Patients Characteristics	Outcomes	Results	Main Findings
Kantas, 2009[26]	Prospective, controlled	IIIb	Symptoms and signs pH monitoring	LPR patients with phonosurgery Reinke's edema (N = 40) Gr 1: PPIs postsurgery Gr 2: no PPIs postsurgery	Postoperative RSI and RFS Epithelialization and recurrence of Reinke's edema	Better in patients of Gr 1 Better in patients of Gr 1	LPR influences epithelization and recurrence of Reinke's edema after partial or total decortication.
Chung, 2009[24]	Prospective, controlled	IIIb	Symptoms and signs pH monitoring	Gr 1: patients with VF benign lesions Gr 1A: Reinke's edema Gr 2: LPR symptoms (N = 200)	LPR prevalence (pH monitoring)	Better in Gr 1A in comparison with Gr 2	LPR is more prevalent in patients with Reinke's edema than in patients with symptoms of LPR. LPR might play a role as an etiologic factor in Reinke's edema.
Katsinelos, 2009[33]	Prospective, uncontrolled	IV	Symptoms and signs pH monitoring	Patients who had GI endoscopy (N = 1130)	Association between GERD and chronic laryngitis findings Association between GERD and Reinke's edema	There was an association between GERD and chronic laryngitis findings. There was an association between GERD and Reinke's edema.	Patients who have demonstrated GERD have a higher risk of chronic laryngitis and Reinke's edema.
Kamargiannis, 2011[34]	Prospective, controlled	IIIb	Symptoms and signs pH monitoring	Patients with Reinke's edema (N = 60) Gr 1: Reinke's edema without LPR (N = 20) Gr 2: Reinke's edema with LPR (N = 40)	RSI RFS Histological chronic laryngitis prevalence	No difference between groups No difference between groups Gr 1: 20%; Gr 2: 50%	Patients with Reinke's edema and LPR according to pH-metry have more histological findings of chronic laryngitis than those without positive pH-metry.
Beltsis, 2011[22]	Prospective, controlled	IIIb	Symptoms and signs RFS >7 pH monitoring	Gr 1: LPR (N = 68) Reinke's edema (N = 12) Gr 2: GERD (N = 24)	LPR episodes (pH monitoring) Patients with Reinke's edema (prevalence)	Gr 1 had higher no. of episodes than Gr 2 LPR (67%), GERD (50%)	LPR is more prevalent in patients with Reinke's edema compared with typical GERD patients.

EL = evidence level of studies according to Oxford criteria; GERD = gastroesophageal reflux disease; GI = gastrointestinal; Gr = group; LPR = laryngopharyngeal reflux; PPIs = proton pump inhibitors; RFS = Reflux Finding Score; RSI = Reflux Symptoms Index; VF = vocal folds.

TABLE V.
Experimental Studies Focusing on Impact of Reflux on Human Vocal Folds.

References	Design	LPR Diagnosis	Sample / Patients characteristics	Outcomes	Results
Axford, 2001[36]	Prospective, uncontrolled	NP	Human vocal folds samples	Expression (IHC, WB) of	
			LPR patients (N = 9)	CA I, II	Positive in LPR patients
				CA III	Negative in LPR patients
Johnston, 2003[37]	Prospective, controlled	NP	Human vocal folds samples	Gene expression (IHC, WB, ISH)	
			Gr 1: LPR (N = 26)	CA I, II	No difference between groups
			Gr 2: CT (N = 19)	CA III	Higher in controls
				E-cadherin	Low expression
				MUC 4 & 5 AC	Higher in controls
Johnston, 2004[38]	Prospective, controlled	Symptoms and signs	Human vocal folds samples	Pepsin tissue level (IHC, WB)	Higher in LPR patients
			Gr 1: LPR (N = 9)	Association between pepsin and CA III depletion	Positive association
			Gr 2: CT (N = 12)		
Gill, 2005[39]	Prospective, controlled	RSI >11 and RFS >5	Human vocal folds samples	Expression of	
		pH monitoring	Gr 1: LPR (N = 18)	Intracellular pepsin (IHC, WB)	Higher in LPR patients
			Gr 2: CT (N = 12)	E-cadherin and CA III expression	Higher in controls
				Association between pepsin and CA III depletion	Positive association
Reichel, 2008[40]	Prospective, uncontrolled	Symptoms and signs	Human vocal folds samples	Expression (IHC) of	
		pH monitoring	Gr 1: pH-metry LPR (N = 14)	E-cadherin	Higher in Gr 2
			Gr 2: clinical LPR (N = 7)	β-catenin	No difference between groups
Johnston, 2010[41]	Prospective, controlled	Symptoms and signs	Human laryngeal samples	Expression (IHC) of	
		RSI 21–30	LPR patients (N = 12)	CD161	Higher in LPR patients
			Healthy (N = 11)	MHC I, II	No difference between groups
				MHC β2m, MHC, CD1d	Higher in LPR patients
Ali et al., 2014[42]	Prospective, controlled	NP	Human vocal folds samples	Gene expression (ISH)	
			Gr 1: LPR (N = 27)	MUC 1, 2, 4	No difference between groups
			Gr 2: CT (N = 3)	MUC3, 5 AC	No difference between groups

CA = carbonic anhydrase; CT = controls; IHC = immunohistochemistry; LPR = laryngopharyngeal reflux; ISH = in situ hybridization; MUC = mucin; NP = not provided; RFS = reflux finding score; RSI = reflux symptom index; WB = Western blot.

only one study assessed the laryngeal exams in a blinded fashion.[33] In the absence of blinded analysis, the evaluation of laryngopharyngeal findings within the included studies is subject to bias; clinical assignment of reflux is known to be influenced by the experience and knowledge of the physician.[49,50] A blinded assessment of laryngopharyngeal findings would provide a more reliable evaluation of LPR findings in a context of clinical researches that study two conditions with some similar findings.

Another difficulty in establishing theories about the origin of BLVF is that LPR diagnosis is investigated in patients presenting with the lesion rather than studied in patients with prelesional states. Supposing that reflux is a major risk factor for the development of BLVF, LPR may mainly occur during the months/years preceding the development of lesions and could be undetectable at the time of the presentation with the lesion in some patients.[51] This point is emphasized by the fact that 25% to 50% of LPR patients have a chronic course of the disease that is characterized by periods of relapse and remission.[2] Better diagnostic methods, perhaps including those that can account for long-term reflux, might help improve future research. For instance, some current histological techniques allow an analysis of the microstructures of the vocal folds, including some microscopic findings suggestive of reflux in patients with suspected prelesional, early, and advanced BLVF.

To better understand the mechanisms of reflux on the mucosa of the vocal folds, many authors conducted experimental studies on human vocal folds. As highlighted in this review, many microscopic mechanisms underlying the development of mucosal changes of the vocal folds have been identified in tissues of LPR patients. Overall, the reduction of defense mechanisms of the vocal folds, the pepsin-induced weakness of the epithelium throughout alteration of cell-to-cell adhesion molecules and intracellular toxicity (chemical phonotrauma), the inflammatory reaction of the tissue, and the related modifications of biomechanical properties may substantially favor creation of mechanical stress and microtraumas during phonation. In addition, hoarseness related to reflux, which is due to modifications of the biomechanical properties of the vocal

folds,[11] may lead to vocal strain and muscle tension, reinforcing a cycle of increased occurrence of microtraumas. As suspected but not demonstrated, these mechanisms could primarily concern for voice professionals who are known to be at risk of LPR with regard to their lifestyle (e.g., stress, food habits) and voice overuse.[52] Figure 2 illustrates the demonstrated and suspected LPR mechanisms that may favor the development of BLVF. From a theoretical point of view based on the current literature, these mechanisms may support the role of reflux in the development of some BLVF such as nodules, polyps, and Reinke's edema. Typically, patients with these lesions have an environment of chronic mucosal irritation that can be associated with throat clearing, chronic cough, hoarseness, vocal hyperfunction, and increased subglottic aerodynamic driving pressures.[53] All of these mechanisms are associated with the occurrence of microtraumas that seem to

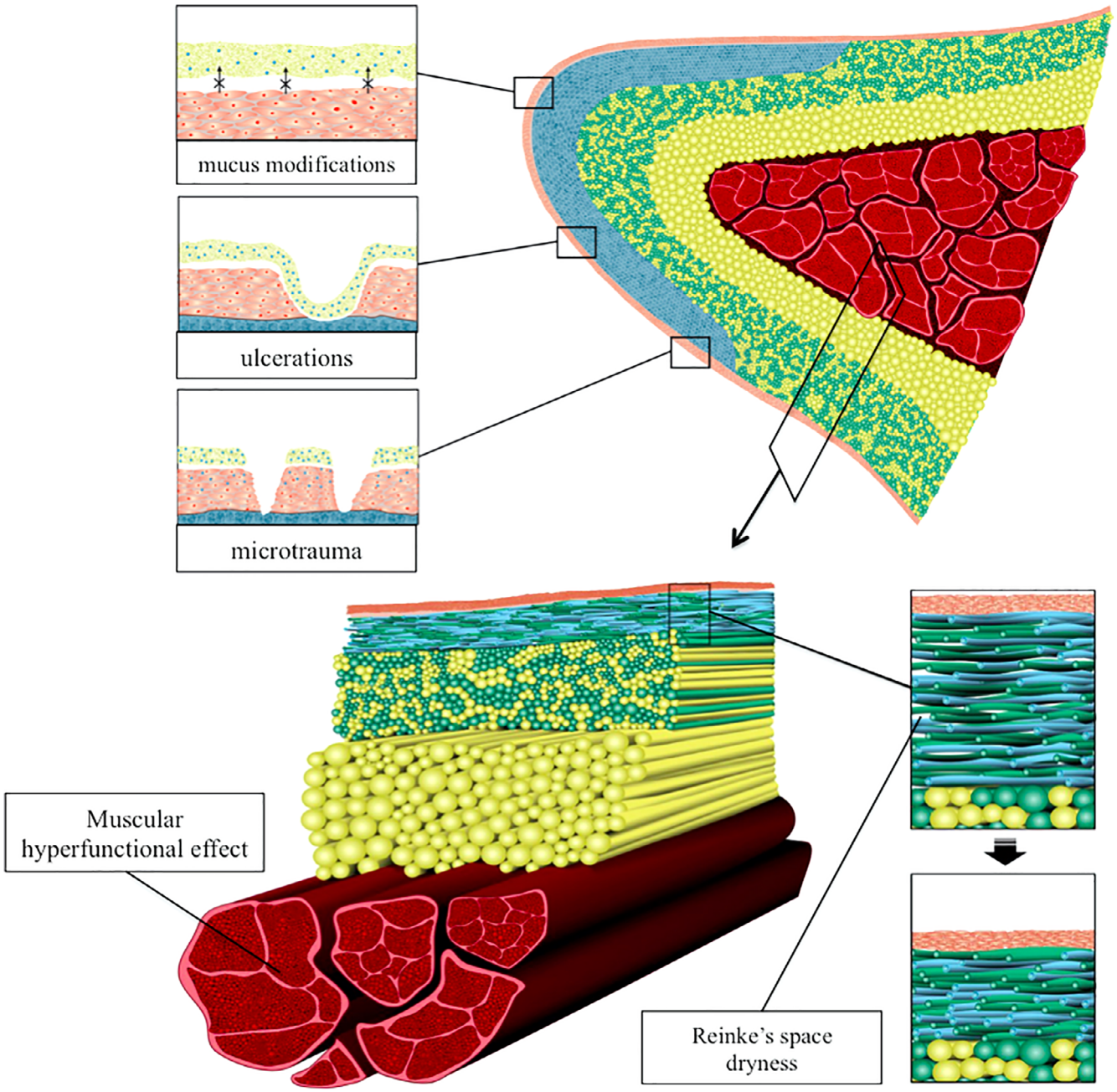

Fig. 2. The pathophysiological mechanisms underlying the development of hoarseness related to reflux and vocal fold benign lesions. The reduction of defense mechanisms of the vocal folds, the pepsin-induced weakness of the epithelium throughout alteration of cell-to-cell adhesion molecules and intracellular toxicity (chemical phonotrauma), and the inflammatory reaction of the tissue and the related modifications of biomechanical properties may substantially favor the occurrence of mechanical stress and microtraumas during the phonation process. In addition, the hoarseness related to reflux, which is due to modifications of the biomechanical properties of the vocal folds, may lead to forcing, strengthening the occurrence of microtraumas.

be a common etiologic factor in the pathogenesis of these three types of BLVF.[54] In the majority of cases, these mechanisms may involve a type of vicious cycle in which the presence of macroscopic and microscopic alterations of the vocal folds makes voice production more difficult, requiring even higher levels of aerodynamic driving forces and myoelastic restoring forces, which in turn further epithelial trauma.

Other factors such as tissue resistance and regenerative capacity can be at play in the development of BLVF in a context of LPR.[26] The healing of the vocal fold epithelium should be impaired by a decrease of the mucosa secretion of epidermal growth factor (EGF).[55] Genetic deficiency of EGF could also be involved in some patients with LPR-associated BLVF, but this hypothesis needs to be proved.[56] In that way, LPR patients could present lower salivary epidermal growth factor concentrations than healthy control subjects, which could strengthen the theory supporting an impairment of the regenerative capacity of mucosa.[57]

About cysts and sulci, review of the literature leads us to be somewhat more reserved regarding the role of reflux in their pathogeneses. At present, no reports have precisely studied the relationship between LPR and the development of epidermoid cysts and sulci vocalis through an analysis of the microstructure of the vocal folds of patients with demonstrated LPR. Instead, the origins of epidermoid cyst and sulcus vocalis could be more complex and involve the interplay of phonotrauma, complex inflammatory reactions,[58] congenital lesions, and possibly a genetic predisposition.[59]

CONCLUSION

To date, it is reasonable to speculate that caustic mucosal exposure from LPR could cause increased susceptibility of the vocal fold mucosa to injury and subsequent nodules, polyps, and Reinke's edema formation, but few studies have been conducted with a low level of evidence and a high heterogeneity between studies about inclusion, exclusion, diagnostic criteria, and clinical outcomes for the diagnosis. Future studies using current diagnostic tools (MII-pH, pepsin, and trypsin detection) and performing tissue analysis of the presence of microscopic findings suggestive of reflux are needed to better clarify the relationship between LPR and vocal fold pathology. Improved knowledge about those factors that favor formation of benign vocal fold lesions will help improve management of these disorders.

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