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Eosinophilic Laryngitis in Children with Aerodigestive Dysfunction

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Abstract

Objective. To describe the presence of laryngeal eosinophils and associated symptomatology in patients with aerodigestive dysfunction.

Study Design. Case series with chart review.

Setting. Single tertiary pediatric referral center.

Subjects. Eighty-one consecutive pediatric patients referred to a multidisciplinary aerodigestive clinic with upper airway concerns.

Methods. Microlaryngoscopy and posterior arytenoid biopsy, flexible bronchoscopy, esophagogastroduodenoscopy and esophageal biopsy, and impedance probe testing were performed as indicated by clinical symptoms. Positive versus negative posterior arytenoid biopsy for eosinophils and the presence or absence of concomitant histopathological laryngitis and/or esophagitis were measured.

Results. Nine of 81 (11%) patients had positive laryngeal biopsy for eosinophils (range, 1-29 eosinophils/high-powered field [HPF]). Three of these 9 patients also had concurrent biopsy-proven eosinophilic esophagitis, while 8 of 81 total patients had biopsy-proven eosinophilic esophagitis. The frequency of biopsy-proven eosinophilic esophagitis was higher in patients with posterior arytenoid eosinophils versus patients without laryngeal eosinophils (33% versus 6.9%, $P = .0408$).

Conclusions. Eosinophilic inflammation in the larynx has not been described in children with complex aerodigestive complaints. Posterior arytenoid eosinophils may serve as a marker of chronic laryngeal inflammation in children with aerodigestive dysfunction, although their exact role in this inflammation remains unclear. In our population, >15 eosinophils/HPF within posterior arytenoid biopsies was associated with concomitant eosinophilic esophagitis.

Keywords

eosinophilic esophagitis, eosinophilic laryngitis, reflux, gastroesophageal reflux, eosinophilia, laryngitis, laryngeal, subglottic stenosis, airway, aerodigestive, laryngeal inflammation, inflammatory, airway reconstruction, dysphagia, aspiration, larynx, pediatric, adult, voice, dysphonia

Pediatric aerodigestive teams have evolved to provide comprehensive, patient-focused evaluation and care of children with diseases of the aerodigestive tract. Sometimes as a cause, and sometimes as a consequence, inflammation of the upper aerodigestive tract is associated with dysfunction in swallowing, breathing, and voicing. While gastroesophageal reflux has long been implicated in inflammation of the upper airway,[1] eosinophilic esophagitis (EoE) is gaining increasing recognition as a contributor to airway edema, asthma, and dysphagia.[2,3]

EoE is a clinicopathologic entity whose manifestations may include feeding difficulties, food impaction, dysphagia, and chest pain. The annual incidence of EoE rose from 1 in 10,000 in the year 2000 to 4.3 cases per 10,000 in 2003,[4] due in part to increasing disease awareness. The diagnostic criteria include upper aerodigestive symptoms combined with >15 intraepithelial eosinophils/high power field (HPF) in 1 or more esophageal mucosal biopsy specimens.[5] Because eosinophils may be increased in the esophagus in the setting of gastroesophageal reflux disease (GERD), the diagnosis of EoE is aided by the exclusion of GERD (although the diseases may coexist).[6] Outside of the esophagus, several small series indicate that chronic rhinosinusitis, asthma, atopy, Eustachian tube dysfunction, sleep-disordered breathing, dysphagia, and airway stenosis are present in patients with EoE, suggesting a systemic inflammatory disorder.[7-12]

Eosinophils have been demonstrated in the distal airway in patients with asthma and within the esophagus in patients with EoE. Animal models have shown increased laryngeal

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eosinophilic inflammation in response to tobacco smoke and allergic stimuli.[13] Greater than a decade ago, eosinophils were discovered in postcricoid mucosa and supra-arytenoid mucosa of children with GERD in 2 studies from separate institutions.[14,15] The density of eosinophils and their histopathologic behaviors were not characterized in those studies. Further, the relationship between laryngeal eosinophils and eosinophilic inflammation in the esophagus has not been explored. The current study evaluates the larynx histopathologically for signs of chronic inflammation and eosinophils in patients with aerodigestive concerns including dysphagia, chronic cough, dyspnea, aspiration pneumonia, airway stenosis, and dysphonia. In the context of a multispecialty aerodigestive team workup, most patients underwent an esophagoscopy (EGD) with biopsy, an impedance/pH probe study, laryngoscopy with biopsy, and bronchoscopy with bronchoalveolar lavage (BAL), a videofluoroscopic swallow study (VFSS), and aeroallergen testing. We provide data from these investigations to characterize patients with laryngeal eosinophils, finding an overall incidence of posterior arytenoid eosinophils to be 11% in our heterogeneous population, with 2 patients exhibiting significant laryngeal hypereosinophilia (>15/HPF) paralleling EoE.

Materials and Methods

After Vanderbilt University Institutional Review Board approval (No. 131846), a retrospective chart review was conducted at a single tertiary pediatric aerodigestive center. Children with aerodigestive concerns who met criteria to be referred to the Complex Aerodigestive Evaluation Team (CADET) at Vanderbilt were evaluated between 2010 and 2013. Outcome measures identified were aerodigestive complaints or findings at presentation including cough, airway stenosis, dyspnea, dysphagia, dysphonia, and recurrent aspiration pneumonia, as well as objective assessments of aerodigestive disease. The latter included results from microlaryngoscopy, posterior arytenoid biopsy, rigid and flexible tracheobronchoscopy, BAL, EGD with biopsies, esophageal impedance testing, aeroallergen testing, select food allergen testing, and VFSS. Posterior arytenoid biopsy was established in 2010 by the aerodigestive team as a clinical standard of care in the setting of upper aerodigestive tract diagnostic endoscopy to screen for upper airway eosinophilia as part of their clinical care.[14] The gold standard diagnostic for GERD was a 24-hour esophageal pH/impedance study, while the gold standard diagnostic for EoE was an esophageal mucosal biopsy indicating >15 eosinophils/HPF. Concerning the esophageal findings, patients were categorized into several groups for secondary analysis: negative impedance/negative esophageal biopsy, positive impedance/negative esophageal biopsy, negative impedance/positive esophageal biopsy, and positive impedance/positive esophageal biopsy. Positive esophageal biopsy for EoE was defined as greater than 15 eosinophils/HPF.

Results

Baseline Clinical Data

Between 2010 and 2013, 81 sequential patients were identified with upper airway concerns who underwent subsequent microlaryngoscopy and arytenoid biopsy for evaluation of laryngeal inflammation. The average age at time of operation was 4.75 years old (range, 4 months–17 years, median, 34 months), and 51 (63%) were boys. Dysphagia was present in 45 of 81 (56%), cough in 40 of 81 (49%), dyspnea in 28 of 81 (34%), aspiration pneumonia in 25 of 81 (31%), airway stenosis in 23 of 81 (29%), and dysphonia in 21 of 81 (26%). Demographic information of the population is summarized in **Table 1**.

Table 1. Demographic Data of 81 Patients.

Boys	51/81 (63%)
Age, y (mean, range)	4.7 years (0.33-16.33)
Cough	40 (49%)
Asthma	20 (25%)
Airway stenosis	23 (28%)
Dyspnea	28 (35%)
Dysphagia	45 (56%)
Dysphonia	21 (26%)
Aspiration pneumonia	25 (36%)
0-2 Aerodigestive complaints	46 (57%)
3-4 Aerodigestive complaints	28 (35%)
5-7 Aerodigestive complaints	7 (9%)
H2 blocker >1 mo	20 (25%)
Proton pump inhibitor >1 mo	39 (48%)
No acid suppression	22 (27%)

Esophageal Impedance Testing

Fifty-six patients (69%) underwent impedance probe testing, and of these patients, 26 (46%) had pathologic acid reflux and 6 (11%) had pathologic nonacid reflux.

Histopathologic Overview

Of the 81 patients who underwent laryngoscopy and posterior arytenoid biopsy, 27 of 81 (33%) had some pathologic sign of chronic inflammation (basal layer hyperplasia, spongiosis, lymphoplasmocytic infiltrate on the pathology report) at the level of the larynx. Nine of 81 patients (11%) had eosinophils within the posterior arytenoid pathologic specimens (range, 1-29; **Figure 1**). Seventy children (86%) also underwent distal and mid esophageal biopsies during EGD. Of those, 23 (33%) had chronic inflammation most consistent with reflux esophagitis, while 8 (11%) had EoE with >15 eosinophils/HPF.

Nine of 81 patients in this series had posterior arytenoid eosinophils. The number of eosinophils per HPF ranged from 1 to 29. Degranulated eosinophils were found in 4 of 9 specimens (both solitary eosinophils that were degranulated as well as degranulated eosinophils in the context of eosinophilic aggregrates). Mild-to-moderate basal layer hyperplasia with mild spongiosis (intracellular edema) of the lamina propria typified the histology (**Table 2**).

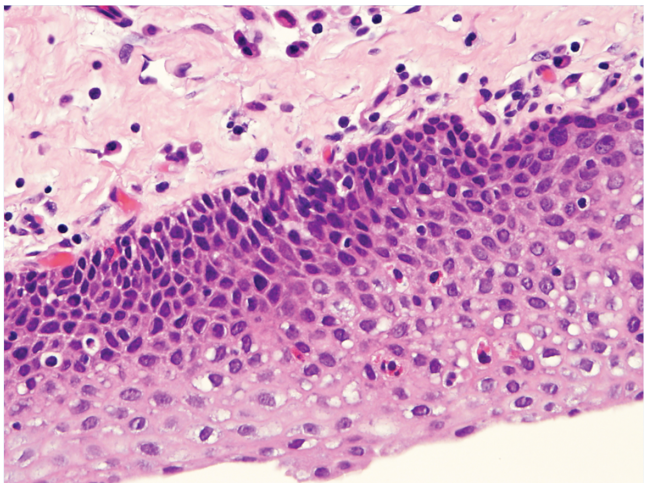

Figure 1. Posterior arytenoid biopsy demonstrating several eosinophils in the lamina propria and within the epithelium in the setting of marked basal layer hyperplasia and spongiosis (60×).

Patients with Laryngeal Eosinophils in Posterior Arytenoid Mucosa

The symptoms at presentation to our multidisciplinary aerodigestive clinic of the 9 patients who were found to have laryngeal eosinophils are listed in **Table 2** along with the results of the multidisciplinary diagnostic effort. From our cohort of 81 patients referred to the aerodigestive clinic, the frequency of biopsy-proven EoE in patients with posterior arytenoid eosinophils, 3 of 8 (38%), exceeds the frequency of biopsy-proven EoE found in patients without posterior arytenoid eosinophils, 5 of 72 (6.9%), $P = .0408$ (Fisher exact test; **Figure 2**). The mean number of laryngeal eosinophils in patients with biopsy-proven EoE was 16.7 versus 1 in patients without EoE. Stated another way, 37.5% (3/8) of patients with EoE had eosinophils in the larynx.

Concerning the lower airway, BAL showed pathologic organisms in the lower airways in 3 of 8 patients who completed flexible bronchoscopy and BAL. In these 3 patients, the organisms, the cell count, and the %neurophils were *Serratia marcescens* (nucleated cell count = 1250, 93% neutrophils), *Serratia marcescens* and *Pseudomonas aeruginosa* (nucleated cell count = 25, 11% neutrophils), and *Haemophilus influenza* (nucleated cell count = 10,780, 95% neutrophils). Five of 8 had upper respiratory flora alone on BAL. The average Lipid Laden Macrophage Index (LLMI) for these patients was 5.25 (median, 6; range, 1-9).

Concerning additional studies, the 3 patients with concurrent EoE all had documented food allergies confirmed through either skin prick or patch testing. Aeroallergens were assessed in 4 of 9 patients, and 1 tested positive for aeroallergens. All 3 patients who had biopsy-proven EoE and posterior arytenoid eosinophils had positive food allergies demonstrated by either skin prick or patch testing. Finally, 8 of 9 patients with posterior arytenoid biopsies positive for eosinophils underwent VFSS prior to airway endoscopy, and 2 of 8 demonstrated frank aspiration.

Discussion

Nine of 81 patients (11%) referred for aerodigestive concerns that included dysphagia, dyspnea, aspiration, and/or airway stenosis had eosinophils in posterior arytenoid biopsies. In addition, 33% of children with eosinophils seen on posterior arytenoid biopsy had concomitant EoE, compared with a 6.9% rate of EoE in the 72 patients who failed to demonstrate posterior arytenoid eosinophils ($P = .0408$). Four of 9 posterior arytenoid biopsies demonstrated degranulated eosinophils with resultant basal layer hyperplasia and spongiosis extending into the lamina propria, allowing for a possible proinflammatory role played by the laryngeal eosinophils. Indeed, 2 patients had 20 and 29 eosinophils/HPF with degranulation and other histological features of inflammation, constituting an eosinophilic laryngitis.

Since 2010, CADET has routinely performed posterior arytenoid biopsies to assess objectively posterior laryngeal inflammation, using this information in the context of esophageal biopsy results to support a diagnosis of esophagitis that has spilled inflammation into the airway.[14] Retrospective analysis of these routinely collected data found 9 patients with laryngeal eosinophils—2 of whom had hypereosinophilia (>15/HPF) to the extent seen in EoE (**Table 2**). Previous studies report the presence of eosinophils in the posterior arytenoid region in humans, but the maximum number of eosinophils per HPF was not reported.[14,15]

In our data set, patients 3 and 5 had 20 and 29 eosinophils/HPF, respectively. Both biopsies demonstrated eosinophils distributed in the lamina propria as well as within the epithelium. Moderate to marked basal layer hyperplasia was noted, as were signs of chronic, inflammatory fibrosis in the lamina propria. Several eosinophils were degranulated in the biopsies from patients 3 and 5. Collectively, these features, with the addition of microabscesses and spongiosis (as seen in patient 3's biopsy), are common histological features of EoE.[16,17] Therefore, we believe the biopsies of patients 3 and 5 are indicative of an eosinophilic laryngitis. Both patients tested positive for food allergy (**Table 2**), and both carry a diagnosis of concomitant EoE. While describing the mechanisms by which eosinophils were attracted to the posterior arytenoid and any damage they may be causing in these patients is beyond the scope of this analysis, certainly, the posterior larynx has intermittent exposure to both food allergens and aeroallergens in sensitized patients. For patients 3 and 5, the observed eosinophilic laryngitis may be mechanistically an anatomically superior manifestation of EoE.

Excluding the increased frequency of EoE observed in patients with eosinophils in posterior arytenoid biopsies, the 9 patients otherwise typified those referred to an aerodigestive team. Perhaps this is in some part due to the diverse effects of eosinophilic inflammation of the larynx, since the products of degranulation are cytotoxic and impair the mucosal sensory function of the larynx, supporting aspiration, and in some part due to the uncontrolled, heterogeneous nature of the population.[18] Two of 9 aspirated on VFSS and the average LLMI was elevated (mean, 5.25),

Table 2. Patients with Posterior Arytenoid Eosinophils.

Patient	Aero Complaint	EoE	Acid Supp >1 mo	Eos/ HPF	Loc of Eos	Degran	Spo	BLH	Incr IE Lymphocytes	Chronic LP Inflam (Non-eo)	Parakeratosis	Allergy Testing (Skin or Patch)	BAL Cell Count	BAL % Neutrophils	BAL LLMI	VFSSPI	VFSS Aspiration (Trace, Frank)
1	Dysp	N	N	1	IE	N	Mild	Mild	N	N	N	NP	NP	NP	NP	N	
2	C, AS, Dysp, DP, AP	Y	PPI	1	IE	N	Mild	Mild	N	N	N	Food	1250	93	6	Y	Frank
3	Asth, DP	Y	PPI	20	IE/LP	Y	Mild	Mod	N	Mild	N	Food	54	2	1	Y	N
4	Asth, AS, Dysp	N	H2	1	IE	N	N	Mod	Mild	Mod	Y	NP	167	20	9	Y	N
5	AS	Y	PPI	29	IE/LP	Y	N	Mar	Mod	Mark	Y	Food	25	11	2	Y	N
6	C, Dysp	N	N	1	LP	Y	N	N	Mild	Mod	N	Negative	48	8	6	Y	N
7	DP, AP	N	PPI	1	IE	N	Mild	Mild	Mar	Mar	N	NP	15	2	6	Y	N
8	C, DP	N	N	1	IE	N	N	Mild	N	N	N	NP	315	73	9	N	
9	DP	N	PPI	1	IE	Y	N	Mild	N	N	Y	NP	10780	95	3	Y	Trace

Abbreviations: AS, airway stenosis; Asth, asthma; AP, aspiration pneumonia; BAL, bronchoalveolar lavage; BLH, basal layer hyperplasia; C, cough; Degran, degranulation; DP, dysphagia; Dysp, dyspnea; EoE, eosinophilic esophagitis; H2, H2 blocker; HPF, high-powered field; IE, intraepithelial; LLMI, Lipid Laden Macrophage Index; LP, lamina propria; Mod, moderate; Mar, marked; N, no; NP, not performed; PPI, proton pump inhibitor; Pt, patient; Spo, spongiosis; Supp, suppression; VFSS PI, videofluoroscopic swallow study preintervention; Y, yes.

Figure 2. Flow chart indicating the prevalence of posterior arytenoid eosinophils and eosinophilic esophagitis during aerodigestive workup of 81 consecutive patients.

although the significance of the LLMI in diagnosing aspiration has been called into question.[19] Three patients had a pathologic organism isolated from the BAL specimen, but only 2 of the 3 had the cytopathologic signature of a true infection (neutrophilia in the nucleated cell count). Two of 3 EoE patients with positive eosinophilic posterior arytenoid biopsies also had BAL cultures that grew pathologic bacteria (both *Serratia* species) versus 1 of 5 non-EoE patients with positive eosinophilic posterior arytenoid biopsies that grew pathologic bacteria (*Hemophilus influenza*). Three had some type of impedance-proven reflux.

Our study is novel in its identification of eosinophilic mucosal inflammation of the larynx. This is distinct from the previously described eosinophilic angiocentric fibrosis of the larynx and may in fact relate more closely to EoE.[20] Accordingly, we found that children with eosinophils in posterior arytenoid biopsies have EoE at a higher frequency than those without eosinophils in posterior arytenoid biopsies in a heterogeneous population of aerodigestive team patients. In addition, in 2 of the 3 patients with posterior arytenoid eosinophilia and concomitant EoE, the number of eosinophils was markedly increased, similar to the pattern seen in the esophagus of patient with EoE. What is unknown is the significance of the posterior arytenoid eosinophils.

There are several limitations to our study. The cohort of 9 patients with posterior arytenoid eosinophils is drawn from a heterogeneous group of children referred to a multidisciplinary aerodigestive team for the diagnosis and management of some aerodigestive dysfunction. A clinic with different inclusion criteria would likely find posterior arytenoid eosinophils at a different frequency. Likewise, the uncontrolled nature of the population does not provide any certainty that >15 eosinophils/HPF in the larynx is associated with EoE. Furthermore, the sample size is small, and a larger, prospective evaluation of posterior arytenoid eosinophilia is currently in progress at our institution, with the hope of answering several critical questions: are cytotoxic

and proinflammatory factors related to pathologic eosinophils present in these larynges, as has been demonstrated in the esophagus in previous studies[21-25]? Is this another site-specific manifestation of food allergy? The present study can neither elucidate the molecular pathophysiology of posterior arytenoid eosinophils nor prove an association between children with specific aerodigestive complaints and posterior arytenoid eosinophils.[26] However, having identified the presence of posterior arytenoid eosinophils—many degranulated with local histological signatures of inflammation—in a substantial series of aerodigestive patients, we hope to further characterize eosinophilic laryngitis as a distinct clinicopathologic entity or as a ''spillover effect'' of EoE that supports chronic laryngeal inflammation and aerodigestive dysfunction.

Conclusion

In 81 patients who presented to a multidisciplinary aerodigestive team, a cohort of 9 of 81 were found to have eosinophils in their posterior arytenoid biopsies. While most patients had a solitary eosinophil, 2 patients had >15 eosinophils/HPF and/or concomitant histological signs of inflammation, constituting an eosinophilic laryngitis. Laryngeal eosinophils may serve as a marker of chronic laryngeal inflammation in patients with aerodigestive dysfunction, and high numbers of eosinophils within the larynx may be associated with EoE.

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Author Contributions

Robert J. Yawn, collection/analysis/interpretation of data, writing of manuscript, revision of manuscript; **Sari Acra**, analysis/interpretation of data, revision of manuscript; **Steven L. Goudy**, analysis/interpretation of data, revision of manuscript; **Raina Flores**, pathologic analysis, interpretation of data, revision of manuscript; **Christopher T. Wootten**, conception/design of study, analysis/interpretation of data, revision of manuscript.

Disclosures

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