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# Abnormal Microvasculature in Laryngectomy Mucosal Margins may be Associated with Increased Risk of Fistula

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## Abstract

Pharyngocutaneous fistula after laryngectomy is common and significantly increases the morbidity of the procedure. Intraoperative, objective variables that can reliably predict fistula formation would be useful to surgeons deciding how to reconstruct the laryngectomy defect. Retrospective chart review of 50 radiated patients and 10 non-radiated patients who underwent total laryngectomy at a single tertiary care institution. Patients with pharyngocutaneous fistula were selected to ensure a representative sample were available for comparison. All patients had pathology slides available for re-review by a single, blinded pathologist. Margins of both radiated ( $n = 50$ ) and non-radiated ( $n = 10$ ) larynges were examined for 7 histologic features, and odds ratios were calculated to assess whether these features were associated with fistula. When evaluating all 60 patients, both telangiectatic capillaries and hyalinized arterioles were associated with fistula (OR 3.72 and 9.21, respectively). Collinearity between the variables was evaluated; findings indicated a high likelihood of having hyalinized arterioles if telangiectatic capillaries were also present (OR 31.67 [3.13, 320.06]). Microvascular changes in radiated tissue have previously been described in other anatomic subsites, but the larynx and pharynx have not been specifically evaluated. Laryngectomy mucosal margins appear to display similar changes, and evidence of this damage may be associated with fistula formation. These features could potentially guide the surgeon to alter the reconstructive technique.

**Keywords** Laryngectomy · Head and neck cancer · Pharyngocutaneous fistula · Radiation

## Introduction

Nonsurgical treatment with radiation or concurrent chemoradiation is an acceptable organ-preserving treatment modality for early stage and selected advanced-stage laryngeal cancer. However, treatment failure or recurrent disease may require that the patient undergo a salvage total laryngectomy. Performing a laryngectomy in a patient who has had prior radiation is associated with a higher complication rate than primary laryngectomy, and an overall complication rate of 67.5% has been reported in the salvage setting [1].

Specifically, pharyngocutaneous fistulas can develop, leading to increased hospitalization time, increased cost, delay in oral feeding, and even a delay in adjuvant treatment [2–5]. While the rate of pharyngocutaneous fistula is typically less than 10% in non-radiated patients, this number can increase to higher than 30% in previously radiated patients [1, 2, 6].

Fistula formation appears to be multifactorial. Some studies in the literature have found an association with advanced disease, poor nutritional status, and postoperative hemoglobin less than 12.5 g/L [2, 5]. However, these risk factors are not consistently associated with fistula, as other multi-center reviews did not demonstrate similar findings [3].

Head and neck surgeons must assess whether operative technique can be adjusted to prevent fistula formation. Many will choose to reconstruct salvage laryngectomy defects with either a pedicled flap or free tissue transfer to introduce non-radiated, healthy tissue to the wound-bed [7]. Patel et al. [3] reviewed their series of patients undergoing a salvage laryngectomy across multiple institutions and noted decreased fistula rates in those who had an onlay pectoralis myofascial

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flap. The authors suggest that this decreased fistula rate is because the interposed flap doubles the suture line where a potential fistula could form, rather than providing superficial reinforcement to the pharyngeal closure [3]. However, not all laryngectomy defects will result in enough native tissue for primary pharyngeal closure with a subsequent onlay flap. Some laryngeal tumors require a resection that includes a partial pharyngectomy, leaving a posterior pharyngeal wall strip of native mucosa to be incorporated in the reconstruction with an interposed flap. If this remaining native mucosa displays radiation damage predictive of worse healing outcomes, it may be better to simply resect this strip of mucosa and reconstruct the resultant total laryngopharyngectomy with a tubed flap.

To our knowledge, the specific histologic effects of radiation in the larynx and pharynx and the subsequent potential effects on wound healing have not been described. However, vascular congestion, telangiectasia, fibrosis, and lymphedema have been described in microscopic evaluation of radiated skin and mucosa [8]. Salivary glands, particularly the parotid, degenerate even after exposure to small doses of radiation, explaining the xerostomia patients often experience [9]. Tumors themselves can also lead to increased fibrin deposition leading to collagen formation and fibrosis [8]. Based on these studies, we chose to assess laryngectomy mucosal margins for telangiectatic capillaries, hyalinized arterioles, dilated lymphatics, sialometaplasia/loss of minor salivary gland mucous cells, fibrosis, thinned epithelium, and radiation fibroblasts or atypical stromal cells. In this retrospective pilot study, we examined whether these histologic changes can be identified in the pharyngeal margins to predict pharyngocutaneous fistula. This may help guide surgeons as to whether the tissue is too damaged to preserve and whether resecting the remaining pharyngeal strip and performing a tubed flap would result in better surgical outcomes.

## Methods

This is a retrospective review of patients who had a total laryngectomy at the University of Miami Hospital and had laryngectomy mucosal margins available for re-review. The Institutional Review Board approved this study (IRB # 20140935). We selected a majority of radiated patients ( $n=50/60$ , 83%) and ensured a large subset had post-operative pharyngocutaneous fistulas ( $n=24$ , 40%) to allow for histologic review of this specific group of patients.

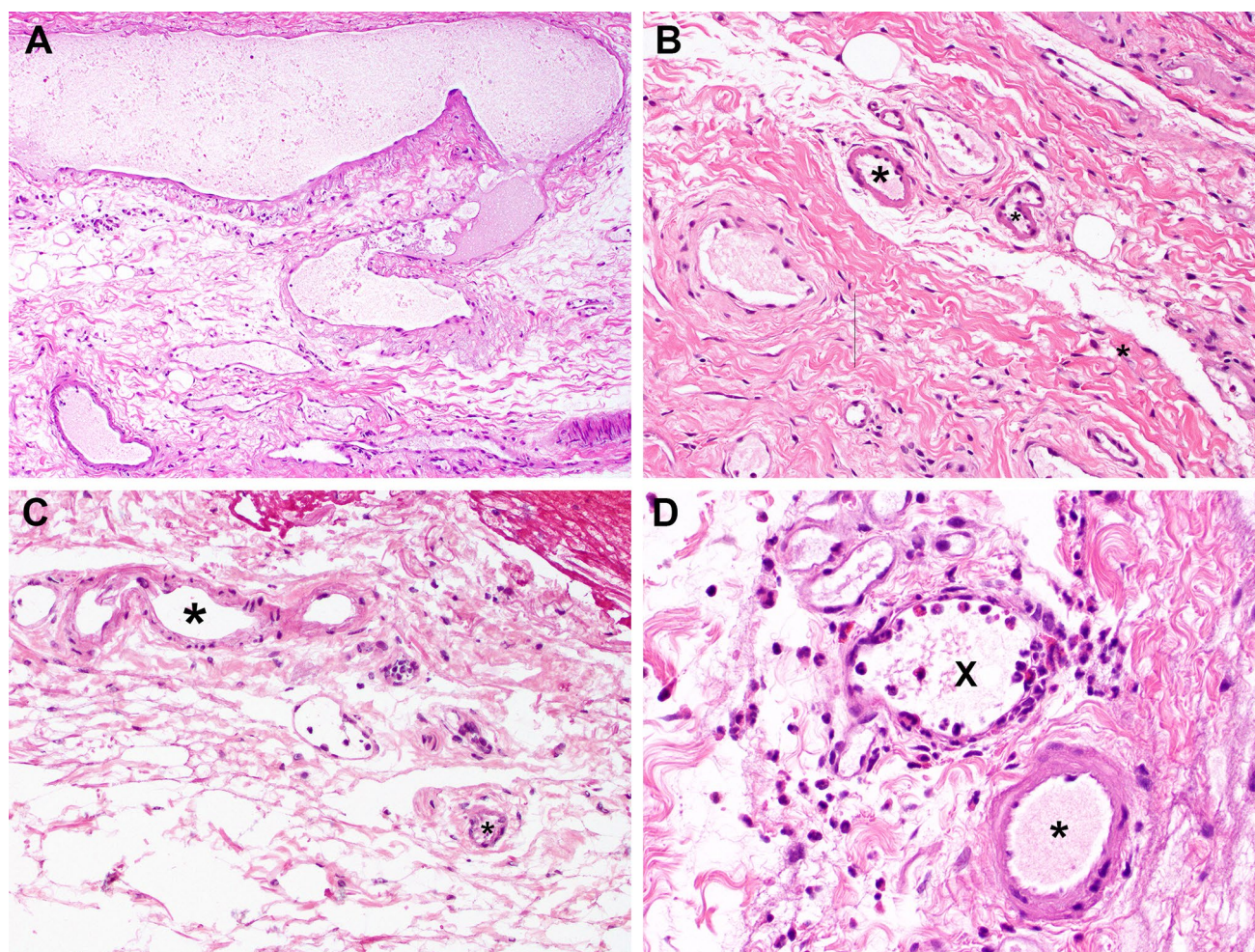
Retrospective chart review was performed to collect demographics and treatment-specific information relevant for analysis for each of the 60 patients. Tumor stage and pre-operative treatment exposure were recorded, as were perioperative hemoglobin, prealbumin, thyroid stimulating

hormone (TSH), and body mass index (BMI), when available.

Hematoxylin and eosin (H&E) section slides were retrieved from all available mucosal margins were obtained and re-reviewed for each patient. A single pathologist (D.K.), blinded to both patient preoperative exposure (radiation, chemoradiation, or none) and postoperative outcome (fistula), reviewed each margin. Margins were assessed for 7 histologic criteria: telangiectatic capillaries, dilated lymphatics, thick-walled or hyalinized arterioles (Fig. 1), submucosal minor salivary gland tissue with sialometaplasia/loss of mucous cells, fibrosis, thinned squamous epithelium, and radiation fibroblasts/atypical stromal cells with enlarged nuclei and smudgy, hyperchromatic chromatin (Fig. 2). Dilated lymphatics were defined as lymphatics (small to large, thin-walled vascular channels with frequently irregular cross-sectional profiles) lined by endothelial cells with uniformly flattened, attenuated nuclei indicative of significant luminal distension rather than plump endothelial cells protruding into the lumen. Thick-walled, hyalinized arterioles were characterized as arterioles (small vessels lined by a layer of circumferentially arranged smooth muscle cells with or without visible internal elastic lamellae) that demonstrated medial expansion by uniform, glassy, eosinophilic material. Telangiectatic capillaries were defined as capillaries (small vessels without a muscular layer or elastic lamellae with regular cross-sectional profiles) lined by uniformly flattened, attenuated nuclei indicative of significant luminal distension. Submucosal minor salivary gland tissue with sialometaplasia/loss of mucous cells was recognized as regions where normal mucinous cells of the salivary gland acini were partially replaced by squamous metaplasia. Fibrosis was considered present when the submucosa demonstrated collagen deposition expanding this compartment. Thinned squamous epithelium was defined as a region of intact (non-ulcerated) squamous epithelium  $<5$  cells thick. Radiation fibroblasts/atypical stromal cells were defined as the presence of cytologically atypical cells with enlarged nuclei, smudgy and hyperchromatic chromatin, and preserved nuclear-to-cytoplasmic ratios.

Each variable was treated as a binary variable, with either the presence or absence of each noted. One to 8 (mean 4) margins were assessed for each patient, as available. If any single margin contained the specific criterion, the patient was considered positive for that criterion. Both radiated and non-radiated margins were evaluated to assess for any differences between these two groups. Subsequently histologic data from patients with fistula or without fistula were compared.

Demographic data were collected and compared between the fistula and no fistula groups to assess for any other confounding variables that may have affected wound healing in our cohort. Histological criteria were assessed for their



**Fig. 1** Vascular histologic features in laryngectomy margins. **a** Dilated lymphatics. Ectatic, irregularly shaped lymphatic channels are prominent within submucosal tissue (Hematoxylin and eosin (H&E), permanent section). **b** Thick-walled, hyalinized arterioles. The arterioles are slightly thickened by eosinophilic material within the media of the vessel walls (H&E, permanent section). **c** Review of the corresponding frozen section margin (from **b**) shows that the

hyalinized arterioles can be detected histologically on frozen section analysis (H&E, frozen section). **d** Telangiectatic capillary (X) adjacent to a hyalinized arteriole (\*). These two histologic features showed collinearity, frequently occurring together, and appeared more commonly in patients with post-operative fistula (effect did not reach statistical significance) (H&E, permanent section)

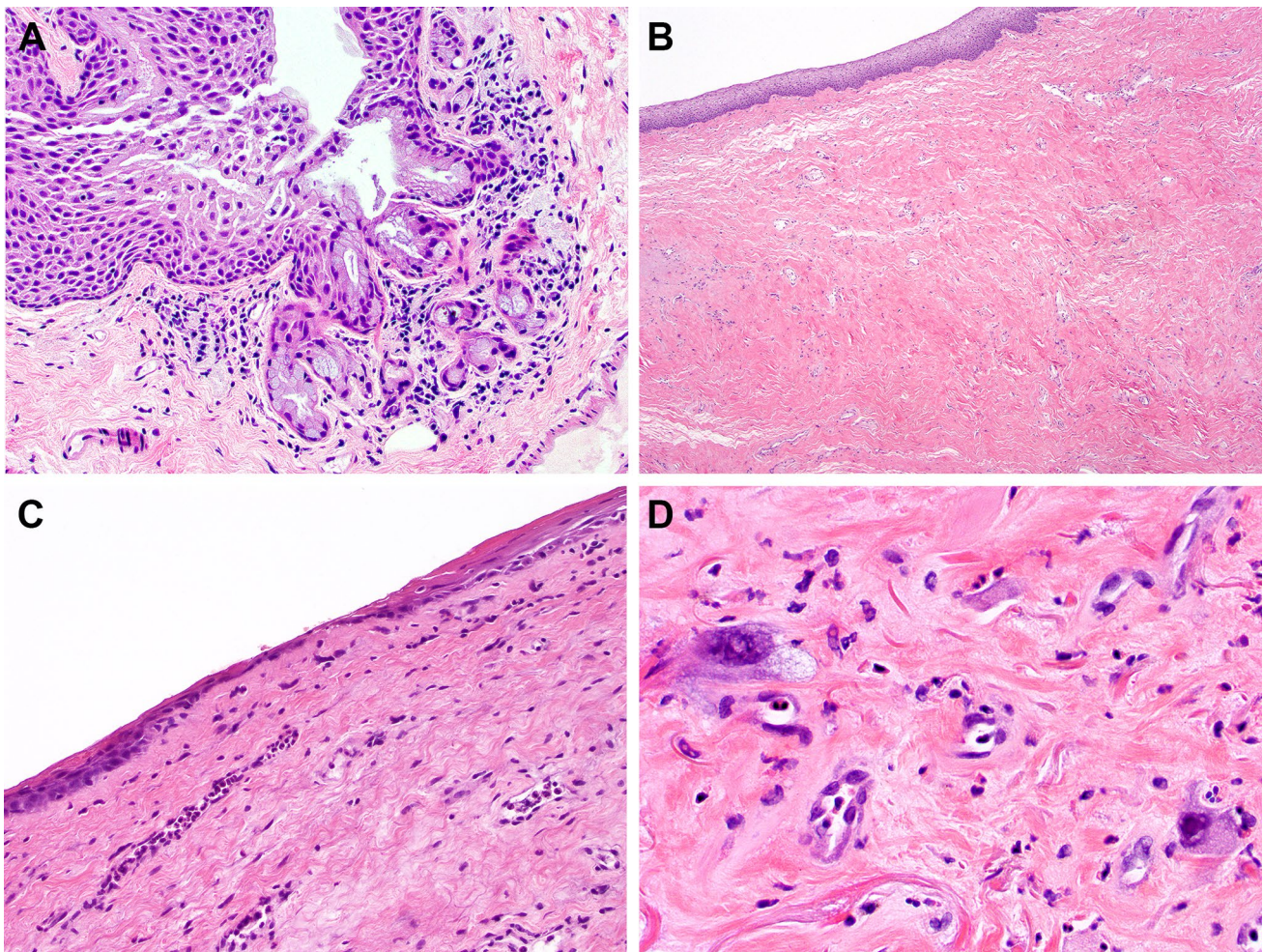
ability to predict fistula formation by calculating odds ratios and performing Fisher's exact tests. Each criterion was also compared to the others to assess if having one criterion predicts that the patient would have another. All statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC), assuming a type I error rate of 0.05.

## Results

The cohort of 60 patients included 54 men and 6 women, ranging in age from 45 to 86 (mean 66 years). Ten of the patients had a primary laryngectomy and 50 had a salvage laryngectomy [after radiation therapy (n = 28) or combined chemotherapy and radiation (n = 22)]. Fifteen patients were

reconstructed with a pectoralis major myocutaneous flap and all 15 patients had prior radiation. Ten patients were reconstructed with a free flap, all had prior radiation. The remaining 35 patients were primarily closed. Additional demographics can be found in Table 1. Perioperative hemoglobin, prealbumin level, TSH level, and BMI were recorded. There did not appear to be any differences in these values when comparing the patients who had a postoperative fistula versus those who did not (see Table 2).

Among the 10 patients who underwent primary laryngectomy, none of the patients' margins displayed hyalinized arterioles or atypical stromal cells with enlarged nuclei, and only 3 patients had margins with telangiectatic capillaries. Dilated lymphatics and fibrosis were present in over 50% of the margins in this group of patients. Table 3 compares



**Fig. 2** Additional histologic features evaluated in laryngectomy margins (H&E, permanent sections). **a** Submucosal minor salivary gland tissue with sialometaplasia/loss of mucous cells. The normal mucinous cells are partially replaced by squamous metaplasia, imparting an atrophic appearance to portions of the salivary gland acini. **b** Fibrosis. The submucosa is expanded with increased collagen deposition, imparting a more densely eosinophilic appearance. **c** Thinned

squamous epithelium. The squamous epithelium ranges from 1 to 3 cells in thickness (normal range 5–25 cells). **d** Radiation fibroblasts/atypical stromal cells. Scattered cytologically atypical cells demonstrating enlarged nuclei and smudgy, hyperchromatic chromatin with preserved nuclear-to-cytoplasmic ratios are present within the submucosa

these 10 patients who underwent primary laryngectomy to a group of 10 patients who underwent salvage laryngectomy; these groups are matched by age (mean 61.5), gender, tumor stage (T3 or T4), and percentage with fistula (30%). This table also shows the entire group of salvage laryngectomy patients. Compared with non-irradiated, primary laryngectomy patients, margins from patients with salvage laryngectomies showed an increase in minor salivary gland tissue with sialometaplasia/loss of mucinous cells, abnormal stromal cells, and fibrosis.

Histologic changes in those who had a postoperative pharyngocutaneous fistula were compared to those with no fistula to see if any of these 7 criteria could be used to predict an increased risk of fistula. Table 4 depicts these findings and shows an increase in microvascular abnormalities

(telangiectatic capillaries and hyalinized arterioles) in patients with a postoperative pharyngocutaneous fistula (OR 3.72 [1.06, 13.05] and OR 9.21 [1.002, 84.67], respectively). Fisher's exact test revealed significantly more hyalinized arterioles in those with fistula ( $p=0.033$ ), and while more telangiectatic capillaries were noted in the fistula group, this did not meet significance with Fisher's exact test ( $p=0.0595$ ). Dilated lymphatics and fibrosis were prevalent in all patients who underwent a laryngectomy. When excluding patients who did not receive radiation, a trend toward increased microvascular abnormalities in the margins of patients developing postoperative fistula was seen, although this did not meet statistical significance. Lastly, collinearity was explored between all variables to assess whether having one variable increased the likelihood that their tissue would

**Table 1** Patient demographics

Age	
Overall	66.4
Primary laryngectomy	64
Salvage laryngectomy	67.38
Prior treatment	
None	10 (17%)
Radiation alone	28 (47%)
Chemotherapy + radiation	22 (36%)
Initial tumor stage (at time of radiation/chemoradiation)	
None	10 (17%)
T1/T2	29 (48%)
T3/T4	13 (22%)
Unknown	8 (13%)
Tumor stage at time of laryngectomy	
T1/T2	5 (8%)
T3/T4	55 (92%)
Patients with pharyngocutaneous fistula	
Overall	24 (40%)
Primary laryngectomy	2 (20%)
Salvage laryngectomy	22 (44%)

**Table 2** Perioperative lab values associated with fistula, reported as mean (range)

Hemoglobin level (g/dL)	
Fistula	12.9 (9.2–14.6)
No fistula	13 (8.5–15.7)
Prealbumin level (mg/dL)	
Fistula	14.1 (4.5–38)
No fistula	13.8 (5–29.1)
TSH level (mIU/L)	
Fistula	11.3 (0.4–87.5)
No fistula	9.2 (0.2–145.8)
Body mass index (kg/m <sup>2</sup> )	
Fistula	24.6 (16.4–35.7)
No fistula	24.8 (16.3–31.8)

**Table 3** Comparing primary versus salvage laryngectomy groups

	Primary laryngectomy (n = 10)	Salvage laryngectomy matched group (n = 10)	Salvage laryngectomy total cohort (n = 50)
Telangiectatic capillaries	3 (30%)	1 (10%)	11 (22%)
Mucous cells with loss of acini	0%	2 (20%)	16 (32%)
Hyalinized arterioles	0%	1 (10%)	6 (12%)
Fibrosis present	5 (50%)	10 (100%)	48 (96%)
Thinned squamous epithelium	0%	0%	5 (10%)
Atypical stromal cells/enlarged nuclei	0%	5 (50%)	30 (60%)
Dilated lymphatics	8 (80%)	9 (90%)	44 (88%)

also display another. Some collinearity was found between telangiectatic capillaries and hyalinized arterioles. Specifically, the margins that had telangiectatic capillaries were also more likely to have hyalinized arterioles (OR 31.67 [3.13, 320.06]).

## Discussion

Salvage laryngectomy for persistent or recurrent cancer after radiation or chemoradiation is the preferred definitive management and can provide patients with the continued ability to eat by mouth and even develop alaryngeal speech. However, wound-healing issues are common and can be devastating to patients, prolonging their hospitalization and delaying functional progress.

The use of free tissue transfers and pedicled flaps has improved outcomes and decreased the rate of pharyngocutaneous fistula, yet a large number of patients still suffer from wound healing problems. This prompted the investigation of whether laryngectomy mucosal margins display varying degrees of damage and whether assessing these factors can predict postoperative fistula. Currently, there is sparse literature examining the histologic changes seen in the larynx and pharynx of patients with laryngeal cancer, regardless of previous treatment history.

Our results histologically examining the margins of 60 patients who underwent total laryngectomy suggest that assessing laryngectomy tumor bed margins for microvascular damage, specifically hyalinized arterioles and telangiectatic capillaries, may prove to be a useful factor in predicting which patients are more likely to have a fistula. The presence of fibrosis was abundant, as expected in radiated patients [8, 9]. In the oral cavity, squamous cell carcinoma cells have been described to express lymphangiogenic ligands and specifically induce the formation of new lymphatic vessels in the tumor microenvironment through a vascular endothelial growth factor pathway [10]. The profusion of lymphatic dilation seen in the current study may thus be related to the presence of tumor rather than radiation damage and may

**Table 4** Comparing fistula versus no fistula groups

	Entire cohort (n = 60)		Radiated patients only (n = 50)	
	Fistula (n = 24)	No fistula (n = 36)	Fistula (n = 22)	No fistula (n = 28)
Telangiectatic capillaries	9 (38%)	5 (14%)	7 (32%)	4 (14%)
Mucous cells with loss of acini	5 (21%)	11 (31%)	5 (23%)	11 (39%)
Hyalinized arterioles	5 (21%)	1 (3%)	5 (23%)	1 (4%)
Fibrosis present	21 (88%)	32 (89%)	20 (91%)	28 (100%)
Thinned squamous epithelium	3 (13%)	2 (6%)	3 (14%)	2 (7%)
Atypical stromal cells/enlarged nuclei	15 (63%)	15 (42%)	15 (68%)	15 (54%)
Dilated lymphatics	21 (88%)	31 (86%)	19 (86%)	25 (89%)

not be a useful criterion in future studies assessing which factors predict fistula.

Finally, previously reported risk factors for wound-healing problems, specifically fistula formation, were examined. Surrogate markers for malnutrition, including prealbumin and BMI, hypothyroidism, and preoperative hemoglobin, were recorded and found to be almost identical between the groups of patients with or without fistula. Thus, further analysis was not performed on these markers.

Our pilot study aims to assess whether histologic evaluation of laryngeal margins can serve as an indicator of tissue health and therefore be useful for predicting subsequent surgical complications, specifically pharyngocutaneous fistula development. To our knowledge, this is the first study to attempt to correlate histologic changes with fistula development in the larynx and pharynx. We assessed margins for 7 histologic features attributed to radiation therapy in prior studies of other organs. One of these features was the structural integrity of the minor salivary gland mucinous cells in the hypopharynx, as these cell types are particularly sensitive to radiation [11]. However, analysis of the margins revealed that mucinous cells were sparse; only 33 of the 60 patients had margins with mucinous cells available for evaluation, making this criterion difficult to analyze. Limitations of our study include sample size, as exemplified by the wide confidence interval, and the fact that we assessed permanent sections of margins submitted for frozen section rather than actual frozen section margins. We assessed permanent sections with the assumption that the higher quality histology seen in these slides would offer the best opportunity to identify the presence of histologic features associated with radiation and would therefore be most useful in judging whether these histologic features are associated with fistula formation. In selected cases, frozen section margins were reviewed and microvascular changes such as hyalinized arterioles could be identified (Fig. 1c). However, future studies are necessary to formally determine whether histologic features that may be important for prognosticating tissue healing can be reliably discerned from the frozen section slides in order to be used for intraoperative decision-making.

Another important limitation is the variability in number of margins available per patient. In a randomized, controlled study, one would want to have a uniform number of margins examined per case with a consistent depth and thickness per margin. However, our retrospective pilot study aims to assess whether histologic examination of margins that are already routinely taken by head and neck surgeons could be useful as an indicator of the tissue integrity or healing capacity. In daily practice, each surgeon takes a variety of margins, but in subsequent, prospective studies, it would be useful to standardize the margin number and depth to allow for a more meaningful analysis.

## Conclusion

This is a pilot study assessing laryngectomy mucosal margins for factors of damage that may affect wound healing. To our knowledge, this is the first study describing histologic findings in this setting and our results show a moderate association between changes identified in the microvasculature of the mucosal margins and patient outcomes. Further analysis with a greater number of both radiated and non-radiated patient specimens will be required to elucidate a stronger, statistically significant association. Ultimately the goal is to develop a grading system to quantify the severity of radiation damage and predict the associated risk of wound-healing problems in that patient that could be utilized at the time of frozen section analysis to potentially modify the surgical procedure.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest to disclose.

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