Lost Voice: A Case Study on Recurrent Laryngeal Squamous Cell Carcinoma Resulting in a Total Laryngectomy and Partial Pharyngectomy

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Abstract:

Laryngeal squamous cell carcinoma (LSCC) is a type of head and neck malignancy that can involve an amalgamation of intricate anatomical features within the supraglottic, glottic and subglottic regions of the larynx. Laryngeal cancer is considered rare compared to other malignancies. Clinical symptoms vary from dysphagia, dysphonia and otalgia to dyspnea, hemoptysis, and globus pharyngeus. LSCC involves the malignant transformation of the laryngeal squamous epithelial lining and has the potential to invade adjacent structures. LSCC is attributed to a history of smoking, alcohol consumption, occupational hazards (i.e. as sulfuric acid mist, wood dust, nickel, etc.), and the **Human Papillomavirus** (HPV). HPV is a sexually transmitted infection that encodes viral oncoproteins which can inactivate important tumor suppressor proteins such as p53 and pRb. Prior studies have found that HPV can cause anogenital or head and neck cancers, appearing decades post inoculation. A multitude of conservative to invasive therapies are available to treat LSCC including chemoradiation and total laryngectomy. The prognosis for laryngeal cancer differs drastically if it is secondary to an HPV infection. Research shows that laryngeal cancers positive for HPV have a better prognosis and respond readily to chemotherapy and radiation compared to HPV negative laryngeal cancers which are more aggressive and typically require more invasive interventions.

Background:

The larynx is vital in providing structural support for the neck, regulating airflow, and preventing the aspiration of ingested materials. The larynx is divided into three regions: supraglottis, glottis, and subglottis. A developing tumor present within these regions can contribute to clinical symptoms of dysphagia, dysphonia, otalgia, and hemoptysis. In general, throat cancer is rare in the United States, representing only 0.6% of all new cancers (1). Common sources of the malignancy can be attributed to tobacco and alcohol consumption, occupational hazards (i.e. sulfuric mist, wood dust, and nickel) or a latent HPV infection.

A 66-year-old male with a 40-pack year history of smoking cigarettes and cigarillos was previously diagnosed with cT3cN0/2 LSCC. The patient completed five months of chemoradiation resulting in residual hoarseness but was in remission. A fiberoptic laryngoscopy and biopsy were preformed three years later to assess the patient's new symptoms of chronic left jaw pain, otalgia, and odynophagia. Immunohistochemistry (IHC) staining was performed to measure the expression of keratin 5/6, p63, and p16 antigens. It was determined that a total laryngectomy with partial pharyngectomy to include the left bottom of tongue (BOT) and bilateral neck dissections would be the last effort to cure the patient's aggressive cancer.

Rationale and Hypothesis:

The leading cause of LSCC is tobacco use and moderate to heavy alcohol consumption, and their synergistic effect drastically increases one's chances of developing laryngeal cancer compared to smoking or drinking alone. It is standard practice to test for HPV via IHC since a latent infection with a high-risk HPV type (16 and 18) is linked to high grade squamous, glandular dysplastic precursor lesions and cancers of the anus, penis, cervix, vulva, vagina, and oropharynx.

IHC staining is routinely performed to measure the expression of p16, a tumor suppressor gene, within mucosal and epithelial carcinomas when evaluating for HPV. A strong and diffuse IHC stain for p16 is indicative of an HPV infection. Studies have found that patients who are HPV-positive respond better to conventional chemoradiation therapy compared to patients who are HPV-negative. A lack of p16 expression is an essential indicator that the malignancy can be contributed to a carcinogen other than HPV.

Methodology:

Strips of tissue from the left pharynx, left pyriform sinus, and left BOT were received in pathology for frozen section analysis. In addition to the total laryngectomy, lymph nodes from levels II and III of the left neck were submitted for pathologic analysis. The total laryngectomy was received fresh and previously oriented with surgical sutures (**Fig. 3**). Included in the total laryngectomy was the larynx, superior trachea with two tracheal rings, hyoid bone, left thyroid lobe, and epiglottis.

Opening the larynx longitudinally revealed a 4.0 x 2.0 cm tan-brown, ulcerated lesion on the left superior aspect of the specimen. Following the College of American Pathologists (CAP) protocols for staging supraglottic tumors, the lesion was sectioned to reflect the relationship to the inferior tracheal ring, left vocal folds, anterior commissure, right vocal folds, right aryepiglottic fold, pyriform sinus, and thyroid. A section map was created to indicate areas of profound interest. The sections were submitted for histological processing and microscopic analysis.

Results:

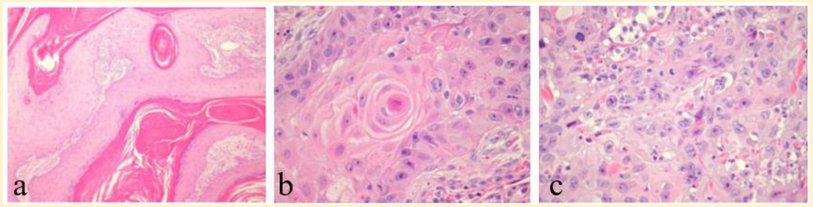

Figure 1. Varying degrees of SCC differentiation depending on recognizable squamous epithelia characteristics such as pink cytoplasm, distinct cell borders, intercellular bridges, pleomorphisms, mitotic activity, and keratin pearls. *a*. well-differentiated, *b*. moderately differentiated, *c*. poorly differentiated. *This figure was not from the patient in this case study but is included to highlight the diagnostic criteria for staging SCC microscopically (Turnball, 2014).*

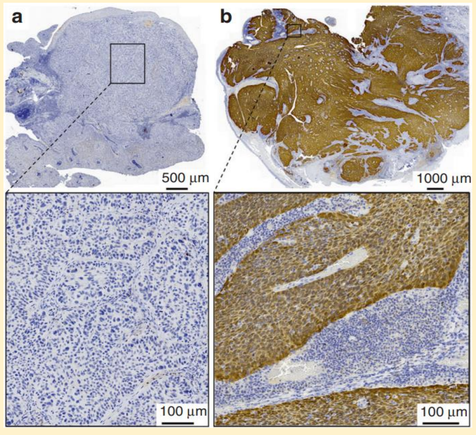

Figure 2. Expression of p16 ^{INK4a} in oropharyngeal SCC detected by IHC staining. **a** No p16 ^{INK4a} expression in OPSCC. **b** Strong, diffuse, overexpression of p16 ^{INK4a} consistent with OPSCC driven by HPV (type 16). *This figure was not from the patient in this case study but is included to compare p16 staining that would be diagnostic for HPV driven malignancy (Wagner et al., 2020).*

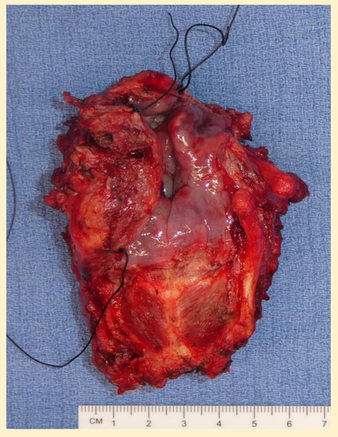

Figure 3. Posterior aspect of a fresh laryngectomy specimen with attached sutures for orientation: *double stitch* - base of tongue, *short stitch* – superior lateral pharyngeal wall, *long lateral stitch* – pyriform sinus.

Microscopic Description and Summary:

The biopsy retrieved during the laryngoscopy revealed the presence of poorly differentiated SCC in the left pharyngeal wall and left BOT. The histological grade was modified to moderately differentiated following the total laryngectomy dissection. The progression to increased cellular disorganization within squamous epithelium (**Fig. 1**) allows the pathologist to accurately gauge the severity of the malignancy.

Tissue samples were sent to an external laboratory for IHC staining of keratin 5/6, p63, and p16 antigens. The results revealed the patient's carcinoma to be immunoreactive for both keratin 5/6 and p63. However, p16 was reported as "**not significant**" in this patient's evaluation. The comparison made in the IHC stains demonstrates negative and positive expression of the p16 gene (**Fig. 2**). In healthy tissue, p16 inhibits cellular proliferation resulting in an absence of expression (a). On the contrary, strong, diffuse, overexpression of p16 indicates that HPV oncoproteins have bound to and inactivated tumor suppressor proteins (p53 and pRb) (b).

Longitudinal sectioning the larynx indicated a 4.0 x 2.0 cm ulcerating lesion in the supraglottis. The tumor grossly involved the epiglottis, left pyriform sinus, and left aryepiglottic fold. The tumor was 0.2 cm from the left deep soft tissue margin and the thyroid cartilage. The margins and the eight lymph nodes submitted were all negative for malignancy. The final diagnosis was moderately differentiated SCC with conventional keratinization staged at pT3N0. Due to the lack of metastatic history during the time of surgery, pM classification was not designated.

Discussion:

Understanding the origin of a patient's pathology offers the opportunity to treat the patient more efficiently, determine a more exact prognosis, and reduce the risk of the disease in other individuals. The American Association of Pathologists' Assistants (AAPA) outlines two grossing techniques for a total laryngectomy. Longitudinal sections are recommended for most carcinomas that are unilateral and transverse sections for sarcomas that are lateral, posterior, or bilateral within the larynx. The CAP cancer staging protocol for laryngeal cancers vary based on the extent of invasion within the three anatomical regions. A supraglottic pT3 tumor, such as the malignancy in this case study, entails that the tumor is limited to the larynx with vocal cord fixation. Additionally, a pT3 tumor is associated with invasion of the postcricoid area, preepiglottic space, paraglottic space, and / or inner cortex of thyroid cartilage.

Cancer cells from the laryngectomy were analyzed using IHC to assess the expression of keratin 5/6, p63, and p16. Keratin 5/6 and p63 are naturally present in the basal and suprabasal layers of keratinized and non-keratinized stratified squamous epithelia. Strong expression of these antigens by IHC in superficial layers of the epithelium, such as the stratum granulosum, could indicate a pathological proliferation. IHC showing diffuse strong nuclear expression of p16 that covers at least one-third of the epithelial thickness is consistent with high-grade dysplasia. Focal staining for p16 in an epithelial tissue sample is consistent with low-grade squamous intraepithelial lesions and indicates either a low-risk or high-risk HPV type. Prior research supports that patients with p16-positive tumors were mostly younger, non-smokers, had a lower T-stage, and were at a higher risk for nodal metastasis when compared to patients with p16-negative tumors.

Accurate staging and effective treatment of LSCC has helped contribute to a 5-year survival rate of 61.5% (4). Recent studies report a steady decline (2-3%) of LSCC over the past 10 years in the United States (1). This can be attributed to a decreased popularity for tobacco use and immunization against HPV. According to the National Cancer Institute, Gardasil 9 became the only FDA approved HPV vaccine in the United States in 2016 for its broad coverage of precancerous and dysplastic lesions caused by certain HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58).

Conclusion:

This case presents the opportunity for pathologists' assistants (PathA) to challenge their gross dissection techniques and knowledge of anatomy. The PathA completes a thorough gross exam for a total laryngectomy by following AAPA Grossing Guidelines depending on the nature of the tumor, creating a section map to help reference the involved pathology occurring, and abiding by the CAP cancer staging protocols which vary amongst the three laryngeal regions. The positive immunoreactivity of keratin 5/6 and p63 from this case revealed a pathological cell proliferation grossly supported by the 4.0 x 2.0 cm ulcerating lesion. Assessing immunoreactivity for p16 through IHC helped to reveal the etiology of the LSCC. The lack of p16 expression indicated that the cell proliferation pathways, p53 and RB1, were not affected by the malignancy. The IHC analysis correlated the anticipated HPV-negative finding given the patient's long history of tobacco use, high tumor staging, and recurrence of malignancy that resulted in invasive surgical interventions.

References:

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