Primary Composite Lymphoma of the Larynx, Composed of Diffuse Large B-Cell Lymphoma and Peripheral T-Cell Lymphoma, Not Otherwise Specified, Presenting as Left Subglottic Tracheal Fistula, Esophageal Diverticulum, and Neck Abscess

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Abstract. Primary laryngeal lymphoma occurs very rarely, accounting for far less than 1% of primary malignant laryngeal neoplasms. To the best of our knowledge, primary laryngeal composite lymphoma has not been reported in the literature. Herein, we report the first case of primary laryngeal composite lymphoma composed of diffuse large B-cell lymphoma (DLBCL) and peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS), in a 43-year-old man. Of special interest is the patient's unique clinical presentation of left subglottic tracheal fistula, esophageal diverticulum, and neck abscess with no discrete mass identified. We describe the clinical and pathological characteristics of this case and review the literature.

Introduction

Squamous cell carcinoma is the most common malignant neoplasm of the larynx. Hematopoietic neoplasms represent less than 1% of laryngeal tumors [1]. Plasmacytoma is by far the most common hematopoietic tumor, followed by non-Hodgkin lymphomas (NHLs). Primary Hodgkin lymphoma, myeloid sarcoma, and mast cell sarcoma are extremely rare at this site [2]. Among NHLs, diffuse large B-cell lymphoma (DLBCL) and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) represent 70-80% of cases [3-4]. Rare cases of NK/T-cell lymphoma, T-lymphoblastic leukemia, γδT-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS) [5-9], have also been reported. To date, primary laryngeal composite lymphoma has not been reported. The term composite lymphoma is defined as "two distinctly demarcated types of non-Hodgkin lymphoma or its rare association with Hodgkin lymphoma within a single organ or tissue."[10]. Herein, we describe the first case of a primary laryngeal composite lymphoma consisting of DLBCL and PTCL, NOS.

Materials and Methods

Immunohistochemical studies for CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD30, CD56, BCL-2, BCL-6, MIB-1, and MUM-1 were performed at the University of Kansas Medical Center. A tissue block was submitted to the Mayo Clinic (Rochester, MN) and immunohistochemical studies for CD21, CD56, LMP1, TIA-1, granzyme B, TCR βF1, TCR γ/δ, and gene arrangement studies for immunoglobulin heavy chain, kappa light chain genes, T-cell receptor gamma and beta chain genes were performed at the Mayo Clinic. Formalin fixed, paraffin embedded tissue sections (4-5 µm) were performed on either DACO stainer using DACO DAB Detection System (Carpinteria, California) or Ventana BenchMark XT using iView/DAB detection system (Tucson, Arizona) with commercially available antibodies according to standard manufacturing protocols (Table 1). All negative and positive controls demonstrated appropriate immunolabeling.
In-situ hybridization study for Epstein-Bar Virus (EBV)-Encoded RNA (EBER) was performed on Ventana BenchMark XT using iView/Blue detection system with a commercially available probe according to standard manufacturing protocols (Table 1). All negative and positive controls demonstrated appropriate labeling.

### Case Report

A 43-year-old Caucasian man presented to a local hospital with three months of progressive dysphagia, weight loss and marked neck swelling. He had a remote history of Hodgkin lymphoma diagnosed in an outside hospital in 1988 and was treated with radiation therapy. He did not have a history of stem cell or organ transplant. However, the serology tests for human immunodeficiency virus (HIV) were not performed. The patient was found to have an esophageal diverticulum and was transferred to our facility due to the CT finding of a retropharyngeal abscess. Initial physical exam in our hospital did not show any lesion in the oral cavity, oropharynx, or neck. Contrast CT scans demonstrated diffuse marked supraglottic, glottic, and esophageal soft tissue thickening and edema as well as left subglottic tracheal fistula and neck abscess (Figure 1). There was no significant lymph node enlargement within the neck. Exploratory surgical exams on the neck showed an inflamed supraglottis and a large esophageal diverticulum with surrounding edema and firmness. No lesions or masses were identified. A neck abscess was also identified, drained, and irrigated. Biopsies from the esophagus and larynx on two occasions showed acute and chronic inflammation with necrosis. The culture from the neck abscess was positive for CO2-dependent Streptococcus and Candida dubliniensis. The patient was then treated with antimicrobial regimens. His hospital stay was further complicated by several rounds of respiratory failure for which he underwent tracheostomy, gastric, and jejunostomy tube placement. The patient subsequently developed left jugular vein thrombosis, pneumonia, and acute renal failure. 18[F]fluorodeoxyglucose (FDG)-positron emission tomography (PET)-CT scan was then performed and showed diffuse increased metabolic activity within the retropharyngeal space and oropharynx extending to the upper mediastinum with a maximum standardized uptake value

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Primary laryngeal composite lymphoma

A SUV of 25.18. Increased metabolic activity with maximum SUV ranging from 3.41 to 17.41 was also seen in the right upper lung, cardia of the stomach, and multiple mesenteric lymph nodes. The possibility of lymphomatous involvement was raised. However, after consulting the hematology service, infectious etiology was still favored. Given that the patient’s larynx was nonfunctional, he was totally dependent on the tracheostomy tube, all possible conservative managements were exhausted, and the primary pathology was still in question, a total laryngectomy and esophageal reconstruction were performed. During the surgery, marked diffuse infiltration of the mid-portion of the neck (from the platysma to the pre-vertebral fascia) with firm, pale tan tissue was identified.

Gross examination of the laryngectomy specimen revealed an eroded mucosal surface with an ulceration obliterating the vocal cords. Histologically, all sections showed a diffuse dense lymphocytic infiltration just beneath the squamous mucosa and involving the thyroid cartilage (Figures 2 A and B) with focal necrosis. There appeared to be two cell populations, one composed of clusters or sheets of large lymphocytes with round to slightly irregular vesicular nuclei, one to several distinct membrane bound nucleoli, and a small amount of amphophilic cytoplasm (Figure 2 C). The second population consisted of small to intermediate lymphocytes, some with a perivascular infiltration pattern. These lymphoid cells had irregular nuclear contours, condensed chromatin, inconspicuous nucleoli, and scant cytoplasm (Figure 2 D).

Due to the clinical impression of infectious etiology, no fresh material was submitted to either flow cytometric study or cytogenetic study at the time of tissue handling. Extensive immunohistochemical studies were subsequently performed. The large neoplastic cells showed strong positivity for CD20 (Figure 3 A) and weak positivity for BCL-6 (Figure 3 B). They were negative for CD5, CD10, BCL-2, and MUM-1 (data not shown). Cyclin D1 was not performed. EBER ISH study demonstrated rare positivity in small and large cells and latent membrane protein 1 (LMP1) immunostain was negative (data not shown). A Ki-67 stain showed a very high proliferation rate of approximately 70-80% (Figure 3 C).

The second population of the small to intermediate lymphocytes expressed CD2, CD3, and CD5 (Figures 4 A-C), with weak or no expression of CD7 (Figure 4 D). The neoplastic cells were predominantly CD8+ T-cells rather than CD4+ T-cells (Figures 4 E and F). They were positive for TIA-1 and Granzyme B (Figures 4 G and H). They also partially expressed TCRβ1 and were negative for TCRγδ (data not shown). These lymphocytes were negative for CD15, CD30, CD56 and CD21 immunostain did not demonstrate any follicular dendritic mesh work (data not shown).
Ki-67 stain again showed an increased proliferation rate of approximately 40-50% (Figure 4 I).

Molecular studies of B-cell immunoglobulin and T-cell receptor (TCR) gene rearrangement performed at the Mayo Clinic demonstrated both clonal immunoglobulin gene arrangement and clonal T-cell receptor gene rearrangement (data not shown). Based on the morphological, immunohistochemical, and molecular studies, the diagnosis of a composite lymphoma consisting of DLBCL and PTCL, NOS was made.

Bone marrow aspiration and biopsy were performed. Both were negative for lymphomatous involvement. The patient was treated with rituximab, cyclophosphamide, hydroxydaunorubicin, Oncovin, and prednisone (R-CHOP) and was on his fourth cycle. Clinically, the patient improved, and repeat PET-CT scan showed considerably improved metabolic activity in the previously described lesional areas. However, several episodes of extensive acute bleeding occurred from his surgical site, which was not responding to embolization treatment, and he succumbed to his condition.

**Discussion**

Primary laryngeal lymphomas are almost exclusively NHLs and the majority of these lymphomas are B-cell lineage. The current case is the first case of primary laryngeal composite lymphoma reported in the literature. Composite lymphoma represents a rare occurrence of simultaneous appearance of two histologically distinct lymphomas in the same anatomic organ [10]. The most reported cases
Primary laryngeal composite lymphoma include a combination of a classic Hodgkin’s lymphoma with a B-cell NHL or rarely with a T-cell lymphoma, two distinct B-cell NHLs, and (less likely) a B-cell NHL and a T-cell lymphoma [15]. Composite lymphomas with B-cell NHLs and T-cell components are rare [15]. The most common B-cell component is DLBCL, followed by hairy cell leukemia, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and splenic marginal zone lymphoma, while the most frequent T-cell components include T-cell large granular lymphocytic leukemia, angioimmunoblastic T-cell lymphoma (AILT), PTCL-NOS, and cutaneous T-cell lymphoma [16]. The underlying mechanisms of the simultaneous presence of two histologically distinct lymphoid neoplasms are not well established. Several theories are proposed. One possibility is that both lymphomas arise from common stem cells with the capacity to differentiate into a B-cell and/or T-cell neoplasm. Genetic mutations in pluripotent cells due to either genetic predisposition or previous exposure to specific therapies or mutagens could result in both clones [16]. Another possibility is the presence of immune dysregulation leading to development of mixed neoplastic lineages. A well-documented example is the composite lymphoma consisting of AILT and EBV-positive diffuse large B-cell lymphoma. In this case, the immune dysregulation present in AILT may favor EBV infection or reactivation, therefore leading to the proliferation and clonal expansion of EBV-infected B-cell clone [16-18]. The association between decreased immune surveillance in CLL patients and the simultaneous presence of clonal T-cell populations in these patients represents another example of the possible role of immune dysregulation in composite lymphoma development [16, 19]. In our case, the patient’s previous history of classical Hodgkin’s lymphoma treated with radiation therapy raises the possibility of both components of composite lymphoma deriving from common progenitor cells undergoing malignant transformation secondary to the radiation regimen. The presence of rare EBV-positive cells detected by EBER ISH study in our case also lead us to entertain the second theory. However, in the previously reported cases of AILT and PTCL, NOS associated with EBV-positive B-cell neoplasm, EBV infected B-cells also expressed LMP1 [18, 20]. Immortalization of infected B lymphocytes has been shown to be induced by EBV latency proteins LMP1 and EBNA2 in vitro [21]. In our case, there were only rare EBER positive cells with no LMP1 expression. Therefore, the second possibility is less likely. This case represents a challenging example from both clinical and pathological diagnostic aspects. The majority of primary laryngeal lymphomas presented with a mass lesion and were found in the

Figure 3. Immunohistochemical stains of the diffuse large B-cell lymphoma component of this composite lymphoma. A. CD20 staining (x400). B. BCL-6 staining (x400). C. Ki-67 staining (x400).
Figure 4. Immunohistochemical stains of the peripheral T-cell lymphoma, not otherwise specified component of this composite lymphoma. A. CD2 staining (x400). B. CD3 staining (x400). C. CD5 staining (x400). D. CD7 staining (x400). E. CD4 staining (x400). F. CD8 staining (x400). G. TIA-1 staining (x400). H. Granzyme B staining (x400). I. Ki-67 staining (x400).
supraglottic region [3]. The initial macroscopic characteristics of these reported cases were usually described either as a smooth mucosal or submucosal mass, whereas only in small numbers of the cases were ulcerated lesions identified [1, 3, 22]. This is the first case of primary laryngeal lymphoma with a unique presentation of left subglottic tracheal fistula, esophageal diverticulum, and neck abscess. The imaging studies of this case were rather unusual as well. CT scans repeatedly reported diffuse soft tissue thickening involving supraglottis, glottis, and esophagus; however, no mass lesion was identified. In contrast, a multi-institutional retrospective imaging study of 20 patients with primary laryngeal lymphoma showed discrete lesions in all cases and 75% patients had unilateral involvement [14]. PET-CT study was available in four of these cases, and all four cases were consistently metabolically active. Similarly, the PET-CT scan in our case demonstrated PDG avidity. However, this was performed at a much later time in the patient’s clinical course.

Likewise, there are several areas of difficulties regarding this patient’s pathologic diagnosis. Multiple biopsies on two occasions were obtained, and all yielded necrotic tissue with mixed populations of inflammatory cells. Though it was very rare for a primary laryngeal lymphoma to present with necrosis [5, 14], there was focal necrosis in our specimen, which obscured the neoplastic nature of the disease. Secondly, the polymorphic appearance of the lymphocytic infiltrates, especially in small biopsies, made it difficult to distinguish a neoplastic lesion from a reactive inflammatory process.

Immunoglobulin and T-cell gene arrangement studies in particular played a pivotal role in making the final diagnosis in our case. Testing with multiple framework region (FR) primers along with adding primers for kappa immunoglobulin light-chain gene arrangement yields a sensitivity from 91 to 98 % and a specificity of 100 %, with good DNA quality [23-24]. Similarly, testing with the full BIOMED-2 primers against TCR gamma (TRG), beta (TRB), and delta (TRD) regions has a sensitivity of 98 % and a specificity of 93 % [23-24]. However, neither of these studies was lineage specific, and morphologic and immunophenotypic correlation was crucial for reaching a final conclusion. In our case, morphologic and immunophenotypic findings in addition to the molecular studies support the diagnosis of composite lymphoma.

Radiation therapy and chemotherapy are the most common and effective treatments for primary laryngeal lymphomas worldwide [3]. Surgery may be necessary as a salvage modality of lymphomas that present with laryngeal obstruction or for tumor debulking. However, surgical excision of a laryngeal MALT lymphoma as the first line of treatment has been reported in the literature [4]. Our patient was treated with R-CHOP therapy.

In summary, this is the first report in the literature of a primary laryngeal composite lymphoma with very unusual clinical presentation and histologic features. Although primary laryngeal lymphoma is very rare, it should be considered in the differential diagnosis of a mass lesion in the neck region or a unique presentation of tracheal fistula, as in our case. PET-CT scan is a sensitive tool that helps to establish the diagnosis.

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References


