Retrospective Evaluation of Diffusion Weighted MRI Imaging of Head and Neck Cancers and the Correlation with Histopathology

THESIS

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Abstract

Oropharyngeal squamous cell carcinoma spreads by local invasion, through the vascular system, and along nerves. While the best prognostic factor is early stage at diagnosis, poor prognostic features include advanced stage, lymphovascular invasion, perineural invasion, anaplastic cells, and non-HPV related etiologies. In the setting of recurrence, a patient has a markedly reduced chance of survival, thus it is pertinent to identify the exact extent of disease to ensure timely and adequate management. This study was conducted to evaluate 1) the relationship of perineural spread on imaging with overall and disease-free survival, 2) the relationship of anaplasia with smoking and 3) consistency among pathologists in diagnosing perineural invasion and radiologists in diagnosing perineural spread. Methodology: A retrospective study was performed evaluating the histopathologic slides and radiographic images of patients with advanced stage oral and oropharyngeal cancers from years 2006-2011. Forty-eight patients had both complete resections and adequate imaging for analysis of perineural invasion and spread. One-hundred and ninety-three patients were histopathologically evaluated for anaplasia, perineural invasion and relationship with smoking and survival. The cases were evaluated with the interpreters blinded to the outcome. Differences in survival were calculated with Log-Rank, Breslow, and Tarone-Ware tests. Chi-squared and two-tailed t-tests were used to evaluate relationships between prospective and retrospective analysis
of imaging and pathology, anaplasia and smoking, and anaplasia and perineural invasion.

**Results:** Perineural invasion on pathology and perineural spread on imaging were identified significantly more frequently on retrospective analysis. Survival differences were noted in cases where patients had involvement of trigeminal, facial, accessory, and hypoglossal nerves. Anaplasia showed no correlation with smoking, regardless of pack-year history, status (current, recent former, remote former, never) or survival. Anaplasia also showed no relationship with perineural invasion. **Conclusion:** Retrospective review of imaging and histopathology revealed significantly more cases of neural involvement of tumor. Specifically, survival distributions were significantly different for trigeminal, facial, accessory and hypoglossal nerves with perineural spread (PNS). Lack of recognition of PNS could result in failure to adequately treat the entire extent of tumor leading to recurrence and potentially death. Interestingly, anaplasia of tumor cells showed no relationship with smoking regardless of amount of tobacco exposure and did not impact survival in this study.
Dedication

This document is dedicated to my fiancé, Christopher, my family and the Bullards for their love, support and encouragement.
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Chapter 1: Introduction

Oral and oropharyngeal cancers affect more than 300,000 people worldwide, accounting for more than 150,000 deaths per year. The etiology of oral and oropharyngeal squamous cell carcinoma (SCC) can be divided into human papillomavirus (HPV)-related and non-HPV related cancers. While the incidence of smoking-related squamous cell carcinomas is declining in developed countries, the incidence of HPV-related SCC is increasing. The prognosis is generally better for HPV-related cancers compared to conventional squamous cell carcinomas, although the best prognostic feature is early diagnosis.

Treatment for head and neck squamous cell carcinoma often involves multiple modalities including surgery, radiation, and chemotherapy depending on the histopathologic subtype and extent of the tumor. While all of these options come with an associated morbidity and mortality, the lack of treatment usually results in death. Five-year survival of locally advanced oropharyngeal cancer varies with HPV status and ranges from 30-60%. Five-year survival for oral cancer is highly dependent on the site of origin with floor of mouth having a much worse prognosis (38% 5-year survival) than tongue (63%) for advanced stage (III and IV). Unfavorable prognostic features such as loco-regional metastasis, lymphatic or vascular invasion, and perineural invasion or spread warrant a more aggressive approach to treatment. Neural involvement is associated with recurrence and/or failure to control disease, which results in further
decreased survival. While surgery and adjuvant therapy remains standard of care for advanced-stage oral squamous cell carcinoma, recent clinical trial outcomes suggest that surgery may not improve survival for patients with advanced-stage oropharyngeal squamous cell carcinoma, data that would favor a nonsurgical organ preservation approach. Given evolving treatment standards, a multidisciplinary approach is often preferred in cases of advanced-stage presentation. Overall prognosis is dependent on proper diagnosis with adequate and timely treatment of all structures involved.

After evaluation for disease extent with imaging (magnetic resonance imaging (MRI) and/or computerized tomography (CT) scans), the surgical approach typically involves resection of the cancer with or without lymph node dissection, and evaluation of permanent sections to determine the need for adjuvant therapy. Histopathologic analysis of the permanent sections can provide diagnostic information such as perineural invasion (PNI), lymphovascular invasion, morphologic subtype, anaplasia and extent of disease. Oropharyngeal squamous cell carcinoma can express focal or diffuse nuclear anaplasia or multinucleation. Anaplasia, derived from ancient Greek, means “backward formation” and refers to undifferentiated cells that bear no resemblance to the mature cell of origin. Lewis et al., defined the parameters of anaplasia as any 40x field with 3 or more tumor nuclei with diameters greater than five times the size of a lymphocyte and multinucleation as any 40x field containing three or more tumor cells with multiple nuclei. In this study, anaplasia and multinucleation were highly related and showed worse survival. In an evaluation of 20 cases of SCC with neck dissection, authors reported cellular cannibalism is associated with anaplasia and invasiveness thus is a positive predictor of aggressive biologic behavior. Cellular cannibalism refers to one
tumor cell engulfing a neighboring tumor cell presumably due to lack of nutrients. In this phenomenon, the engulfed cells dies of starvation in contrast to the result of phagocytosis where the engulf cell is lysed. While it is known that cancer DNA is passed vertically (as a cell divides), studies in rat models suggest that the oncogenes present in an engulfed cell can be instituted into the cannibalistic cell’s DNA, leading to further mutations. These cannibalistic cells are resistant to low pH and their presence has been associated with worse prognosis.

Another major prognostic feature of squamous cell carcinoma is the extent of perineural invasion (PNI) and perineural spread (PNS). In addition to worse prognosis, PNI is associated with an increased risk of recurrence, higher morbidity and mortality. While sometimes used interchangeably, the two entities are distinct. PNI refers to the histopathologic evidence of tumor around or within a nerve whereas PNS refers to evidence of tumoral spread along nerves away from the primary tumor site noted on imaging studies or clinically.

Squamous cell carcinoma spreads by invading through the basement membrane structure of the epithelium and surrounding the lymphatic vessels, blood vessels, and nerve structures. Squamous cell carcinoma is known to spread along nerves, especially cranial nerves V and VII. Although the pathophysiology of PNI and PNS is not completely understood, new studies suggest that malignant cells secrete factors that facilitate PNI and PNS. In a 2014 review by Roh et al., factors previously evaluated for their role in PNS of tumors included brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotropin-3 and neurotropin-4, glial cell-line derived neurotrophic factor (GDNF), neural cell adhesion molecule (NCAM), substance P (SP),

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nerve growth factor receptor (NGFR), chemokines, and other factors such as matrix metalloproteinases (MMPs). Notably, NGF and members of the tyrosine kinase receptor (Trk) family were reportedly expressed at higher levels in SCC specimens positive for PNI. Recent studies in squamous cell carcinoma of the skin have identified specific genes in PNI-positive cancers that are absent in PNI-negative cancers; however, reliable markers of PNI remain elusive.

The incidence of PNI in squamous cell carcinoma has been reported in the range of 27-36%. Invasion into the layers of nerve tissue defines PNI, although the universally accepted criteria is somewhat vague. Perineural invasion was defined by Liebig et al as tumor in, around, and through any of the nerve layers (epineurium, perineurium, endoneurium) requiring at least one-third of the nerve to be encapsulated by the tumor or tumor in close proximity to a nerve. While the presence of PNI affects the course of treatment, it is often underreported. Kurtz et al reported that upon re-review of the same slides the presence of PNI found increased from 30% to 62% and that false positive and false negatives were common. Thus, presence or absence of reported PNI by the pathologist can vary due to sampling error, expertise, use of immunohistochemical stains, and the definition of perineural invasion. Further support of the lack of agreement among pathologists was shown in a recent study performed by Chi et al. This study evaluated inter-pathologist concordance and found that identification of PNI was variable with the greatest discrepancy in cases where the tumor was in close proximity to the nerve.

Some authors suggest that disease-free survival is correlated with extent of perineural invasion relative to tumor edge. Miller et al. evaluated prognosis as a function
of negative, intratumoral, peripheral, or extratumoral nerve involvement. While negative and intratumoral involvement outcomes trended toward increased disease-free survival when compared to outcomes in cases with peripheral and extratumoral PNI, the results were not statistically significant (p=0.07). Interestingly, no statistically significant difference in the size of nerve was found.25 Since assessing tumor extent based on histopathologic findings can be unreliable, evolving imaging techniques and technology may be helpful in more accurate determination of pretreatment neural extension of lesional tissue. 9, 21, 27, 39-41

Magnetic resonance imaging (MRI) previously known as nuclear magnetic resonance imaging uses the presence of hydrogen atoms (H+) found in the water and fatty tissues of the body to produce a three-dimensional image. Because hydrogen atoms have an odd atomic number of just one proton and one electron with no neutron, they behave like a small spinning bar magnet, with a positive and negative pole. All of the hydrogens found within the body either in water (H₂O) two hydrogens with one oxygen, or in fat, such as oleic acid (CH₃(CH₂)₇CH=CH(CH₂)₇COOH, a carbon with long fatty chain of hydrogens, randomly spin in the body, with a various axis, for each one. When placed in an external magnetic field, such as one produced by an MRI scanner, all the protons (hydrogens) line up with the direction of the magnetic field with positive and negative charge aligning with the external magnetic field’s positive and negative charge, creating a uniform alignment of all the hydrogens referred to as the net magnetic vector oriented with the axis of the magnetic scanner’s field. The spinning of a proton in an external magnetic field is determined by the Larmor equation, \( \omega = \gamma B \), where \( \omega \) = the Larmor
frequency in MHz, and \( \gamma \) is the gyromagnetic ratio in MHz/Tesla which is a constant specific to each nucleus, and B is the strength of the magnetic field in Tesla. As a result, each proton spins with a constant frequency when exposed to an external magnetic field. For example, all hydrogens when exposed to a magnetic field of one Tesla, spin with a frequency of 42.58 MHz. When a radiofrequency pulse is applied with the same frequency of 42.58 MHz (or resonance) the net magnetic vector of the protons is changed. When the strength of the magnetic field is varied slightly by a gradient, for example one side is 1.5 the other .95 Tesla, each proton will spin with a specific frequency constant to the field exposure and can now be localized in space. After a radiofrequency pulse is applied that flips the protons from aligning with the field, is turned off, the protons will return to their initial net magnetic resting state, aligning with the external magnetic field. In doing so, the protons emit a radio-wave signal that is picked up a receiver coil which is used to generate an image in MRI, with the intensity of the signal plotted to a grey scale on cross-sectional images. Multiple radiofrequency pulses may put in different sequences to focus on different types of tissue, because tissues relax at rates that differ from each other, once the radiofrequency pulse is turned off. The time it takes for the magnetic vector of the hydrogens to return back to the resting state is called T1 relaxation, and for the axial spin to its resting state is called T2 relaxation. So for example when a T1 weighted pulse sequence with a radiofrequency pulses, because hydrogens in a long fatty chain align back rapidly with the magnetic field, they will give a bright signal on a T1-weighted MRI, versus the protons in water which align more slowly and appear dark on a T1-weighted MRI. By delivering pulses, the machine flips the hydrogens from their normal resting state, and as they return to that resting state they
emit a signal picked up by receiver coils. The time it takes the signal to go back to the resting state differs among various tissues. In T1 weighted imaging, fatty tissues with hydrogen chains align rapidly compared to the hydrogen in the water-filled tissues like cerebrospinal fluid, and, fat will appear brighter than water. Inversely, in T2 weighted imaging, the hydrogens which are measured in the transverse plan will be the highest in those that are slowest to leave this plane and align with the external magnetic field, thus, water is brighter than fat on a T2 weighted image. This sensitivity of MRI for delineating tissue differences is important for evaluating tumors.

When evaluating tumor, the appearance of the tumor edge or margin is critical. Thus, tumor surrounded by a fat plane suggests a less aggressive course compared with a tumor with hazy margins or no visible fat plane. Diffusion weighted MRI allows the radiologist to evaluate normal water versus pathologic water in cells. Contrast media such as gadolinium provide better visualization of the disease and its relationship to surrounding structures.

The sensitivity of MRI detection of PNS with a 3.0 Tesla magnet was found to be 95% and as well as being able to predict the zonal extent of the tumoral disease. Radiographic features of PNS include asymmetric enhancement or thickening of the nerve, secondary fatty denervation changes of the muscles of innervation, obliteration of the fat planes, and an enlarged foramen or fossa. Recent techniques including the use of 3 Tesla magnet MRI have shown promising results with sensitivity of 95% and specificity of 84% in properly identifying neural involvement by tumor.
Tumors of the head and neck can spread along any of the cranial nerves in its path and can travel in an antegrade or retrograde direction. That is, the tumor can track away from the brain (antegrade) or towards the brain (retrograde). The most commonly affected cranial nerves in head and neck cancer are CN V and VII, necessitating radiologic review of the entire neural pathway from origin to distal branches. To adequately assess extent of PNS on imaging, complete understanding of the neural tracts must be obtained.

Normal anatomy:

Trigeminal (V1, V2, V3)

V1- ophthalmic branch

V2- maxillary branch palatine to pterygopalatine fossa

V3- mandibular branch including auriculotemporal, lingual

The Trigeminal nerve (CN V) exits the brainstem at the pons and travels through Meckel’s (trigeminal) cave before it splits into three main divisions: ophthalmic nerve (V1), maxillary nerve (V2) and mandibular nerve (V3). Together, these divisions provide sensation to the face and scalp and motor innervation to the muscles of mastication, tensor tympani (of middle ear) and tensor veli palatini (of soft palate). From the trigeminal ganglion, the (V1) ophthalmic nerve, the smallest division of the trigeminal nerve extends anteriorly to enter the superior orbital fissure, supplying orbital structures, the eye and ethmoidal sinuses before the frontal nerve branch exits through the supraorbital notch and supratrochlear foramen. The maxillary nerve (V2) enters the pterygopalatine fossa from the foramen rotundum. This nerve reportedly accounts for the
highest prevalence of PNS in head and neck cancers.\textsuperscript{22} The maxillary division provides sensory innervation for the area of the face overlying the maxillae, the nasal cavities and paranasal sinuses, and mid-face regions. From the fossa, some V2 fibers travel anteriorly and inferiorly to innervate the maxillary teeth. Other V2 fibers travel through the pterygopalatine ganglion and greater and lesser palatine foramina to become the palatine nerves that innervate the hard and soft palate. Another branch of V2, the infraorbital nerve, travels through the infraorbital canal to innervate the lower eyelid, upper lip and part of the anterior aspect of the nasal cavity. The mandibular nerve (V3) has both a motor and sensory component, the sensory division of V3 enters the trigeminal ganglion, however the motor root of V3 bypasses the trigeminal ganglion and exits via foramen ovale, and unites with the sensory root that also extends through foramen ovale, just below foramen ovale descending between the tensor veli palatini muscle and the lateral pterygoid muscle, the motor root then supplies the muscles of mastication, the medial and lateral pterygoids, masseter and temporalis muscles and the sensory root then gives rise to the auriculotemporal nerve, lingual nerve, and inferior alveolar nerves.\textsuperscript{45}
Figure 1. Trigeminal nerve (CN V) arising from the pons and dividing into ophthalmic (superior), maxillary (middle), and mandibular (inferior) nerves.
Facial (VII, chorda tympani, geniculate ganglion, vidian nerve)

The Facial nerve (CN VII), has multiple functions including a general sensory afferent, the posterior auricular nerve, a special sensory afferent, the chorda tympani nerve, a branchial motor for the muscles of facial expression and a visceral motor supplying the parasympathetic innervation to the greater superficial petrosal nerve that extends through the geniculate ganglion and joins with the sympathetic deep petrosal nerve, to form the vidian nerve, which then enters the vidian canal. The nerve extends in
the cerebellopontine angle cistern through the internal acoustic meatus and travels through the facial canal within the petrous ridge. At the first anterior bend, or geniculate ganglion gives off the greater superficial petrosal nerve (parasympathetics) and joins with the deep petrosal (sympathetics from the carotid plexus) to form the vidian nerve (nerve of the pterygoid canal). Just before the Facial nerve exits via the stylomastoid foramen, the chorda tympani passes through the middle ear and exits via the petrotympanic fissure to provide parasympathetic innervation to the submandibular ganglion and sensation of taste from the anterior 2/3 of the tongue. The Facial nerve contains posterior auricular, temporal, zygomatic, buccal, and cervical branches that provide motor function to many muscles of facial expression.
Figure 3. Facial nerve exiting stylomastoid foramen and dividing into five main branches.

Glossopharyngeal, Vagus and Accessory nerve

The Glossopharyngeal nerve (CN IX) exits the skull base through the jugular foramen with the Vagus (CN X) and Accessory nerve (CN XI). The Glossopharyngeal then travels to the posterior oropharynx providing motor innervation to the stylopharyngeus muscle and sensory innervation to the soft palate, posterior tongue, pharynx, tonsils and portions of the larynx. It also provides sensation of taste from the posterior 1/3 of the tongue and parasympathetic innervation of the parotid gland via the otic ganglion. The Vagus nerve travels caudally providing innervation to the muscles of
the pharynx, sensation from inferior portions of the larynx, innervation to the intrinsic muscles of the larynx, and via the main nerve travelling in the carotid sheath, parasympathetic innervation to the thorax and abdomen, as well as pain, temperature and sensory innervation along its tract. The Accessory nerve has both a cranial and a spinal root that exit via the jugular foramen. The cranial root joins with the Vagus to innervate muscles of the pharynx and the spinal root innervates sternocleidomastoid muscle and trapezius muscle.

Figure 4. Jugular foramen shown with Glossopharyngeal, Vagus, and Accessory nerve passing through.
Hypoglossal nerve

The Hypoglossal nerve (CN XII) exits the skull base through the hypoglossal canal. It travels laterally and inferiorly until the level of the angle of the mandible, where it turns medially and anteriorly to enter the base of the tongue bilaterally, and innervates the muscles of the tongue. Damage to CN XII can result in deflection of the tongue to the side of involvement on protrusion.

Figure 5. Hypoglossal nerve exiting skull base through hypoglossal canal.
Objectives:

The overall objective of this study is to retrospectively review the imaging studies and histologic specimens of head and neck squamous cell carcinoma for evidence of perineural invasion and/or perineural spread.

Specifically, this study will:

1. Review pretreatment imaging (CT, MRI, PET) for tumor involvement of cranial nerves, including Trigeminal and its three major divisions (V1, V2, V3), its branches palatine (V2) and auriculotemporal (V3), Meckel’s (trigeminal) cave, Facial nerve (CN VII) including geniculate ganglion, chorda tympani and vidian nerve, Glossopharyngeal (CN IX), Vagus nerve (CN X), Accessory (CN XI), Hypoglossal (CN XII), cervical lymph nodes, extracapsular spread, and skull base.

2. Examine the impact of perineural invasion on disease-free and overall survival.

3. Compare initial and retrospective findings of PNI/PNS for both imaging and histology.

4. Evaluate the association of smoking with anaplasia, anaplasia with perineural invasion, and anaplasia with overall survival.

Hypotheses:

- We hypothesize that perineural invasion and perineural spread is underreported and subsequently adversely affects the survival of advanced stage head and neck squamous cell carcinoma cancer patients.
● We hypothesize that tumor cell anaplasia is directly correlated with the amount of tobacco exposure.

● We hypothesize that risk of recurrence and/or death is greater in patients with major nerve involvement.
Chapter 2: Materials and Methods

This study was approved by The Ohio State University Institutional Review Board (protocol number 2001C0035) as an expedited review. Two-hundred and ninety-three patients with head and neck squamous cell carcinoma were identified; however, based on exclusion criteria, eligible subjects were reduced to 193. Exclusion criteria included lack of resection, unavailable slides, resections performed outside of The Ohio State University, nondiagnostic outside imaging, and multiple malignancies of different origin. Patients with only post-operative imaging or only one type of pretreatment imaging (CT, PET or MRI only) were also excluded. PHI such as age, sex, age at diagnosis, year of diagnosis, tobacco history, subtype of OPSCC, disease-free survival (DFS), overall survival (OS), and months of follow-up was collected. Smoking categories included pack-years, yes/no, further subdivided into current, recent-former (quit within 10 years of diagnosis), remote-former (quit more than 10 years before diagnosis), and never smoker. Each case was subsequently de-identified and assigned a study number (1-293 prior to exclusions) for privacy protection and investigator blinding. Personal health information (PHI) such as age, sex, age at diagnosis, year of diagnosis, tobacco history, subtype of SCC, disease-free survival, overall survival, and months of follow-up was collected. Smoking categories included pack-years, further subdivided into current, recent-former (quit within 10 years of diagnosis), remote-former (quit more
than 10 years before diagnosis), and never smoker. Race was not included as it could not be accurately assessed for all patients. Each case was subsequently de-identified and assigned a study number (1-293 prior to exclusions) for privacy protection and investigator blinding.

**Patient Identification and Sampling**

An electronic database review of medical records from The Ohio State University Wexner Medical Center for advanced stage oral and oropharyngeal cancer during the years 2006-2011 was performed. Resected specimens for all cases (N=193) were reviewed retrospectively for microscopic evidence of PNI and anaplasia. Perineural invasion included tumor within, around, or through a nerve, in close proximity to a nerve with at least 33% of the nerve surrounded. “Close PNI” was assigned in cases where PNI that was suggestive but not definitive, or if tumor was near a nerve but not encircled by at least 33%. This separate category included positive PNI and cases that did not fit the definitive criteria of PNI. Anaplasia was defined according to Lewis et al criteria as positive if three or more cells five times that of a lymphocyte were present within one 40X field.\textsuperscript{15}

The second data group that was analyzed included those of the previous set who had adequate imaging studies (MRI, CT, PET). Of the 193 patients, 145 were excluded for lack of comparable imaging, leaving forty-eight patients who underwent complete resection with slides available for analysis.
Histopathologic analysis

Each specimen was examined in its entirety for perineural invasion and anaplasia including all margins, lymph nodes, and salivary glands. Photomicrographs of representative cases were taken to document presence of perineural invasion and anaplasia using MicroPublisher 5.0 RTV. The photomicrographs of anaplasia were taken at 40x magnification and the magnification of the neural invasion varied depending on the size of the nerve and ranged from 4x to 40x. All magnifications were recorded.

Figure 6. Representative photomicrograph of squamous cell carcinoma surrounding multiple nerves. H&E 10X
Radiographic analysis

Each imaging series was reviewed by a board-certified neuroradiologist starting with the earliest pre-treatment series. The cases reviewed site of initial tumor and involvement of the following structures: cervical lymph nodes, extracapsular spread (ECS), Meckel’s cave (trigeminal cave), Trigeminal (CN V) nerve and its divisions (V1- ophthalmic, V2-maxillary including palatine nerve and pterygopalatine ganglion and fossa, V3-mandibular including auriculotemporal nerve), Facial (CN VII) nerve and its branch chorda tympani, geniculate ganglion, vidian nerve (nerve of the pterygoid canal), Glossopharyngeal nerve (CN IX), Vagus nerve (CN X), Spinal Accessory nerve (CN XI), Hypoglossal nerve (CN XII), vidian nerve (nerve of the pterygoid canal), and skull base. Perineural spread criteria included asymmetric enhancement or thickening of the nerve, secondary fatty denervation changes of the muscles of innervation, obliteration of the fat
planes, and an enlarged foramen or fossa. Images were reviewed in all planes (axial, sagittal, coronal).

**Figure 8. Axial view representation**

**Figure 9. Sagittal view representation**
**Statistical analysis**

Overall and disease-free survival distributions were plotted using Kaplan-Meier plot for perineural spread for each cranial nerve (V, VII, IX, X, XI, XII) and anaplasia. Differences in the distribution were tested with Log-Rank, Breslow, and Tarone-Ware. The Log-Rank test shows differences in survival distribution at each point in time. While it is useful, it can result in a statistically significant difference in survival with low power. Breslow was used to identify early differences in survival. As seen in the data, a larger p value from this test compared to the other two tests suggests that differences in survival were occurring later. Tarone-Ware test was used to show survival differences in the middle. Chi squared tests were used to determine differences between the initial report and retrospective review for both perineural invasion on pathology and perineural spread on imaging studies. A chi-squared test was used to evaluate the relationship between anaplasia and smoking and anaplasia and perineural invasion, and a
two-tailed t-test was used to determine if there was a relationship between anaplasia and tobacco exposure (by total pack-years).
Chapter 3: Results

Perineural spread (PNS)/Perineural Invasion (PNI) Data Results:

Forty-eight patients met inclusion criteria. Thirty-eight were male and 10 were female. Ages ranged from 35 to 74 years with a mean age of 56 years. Histologically, all patients had squamous cell carcinoma. Survival analysis using Kaplan-Meier method was performed to observe trends with overall survival and disease-free survival, relative to the initial evidence of perineural invasion, retrospective evidence of perineural invasion (including a category that included tumor near nerves that could not be called with certainty), initial radiographic evidence of perineural spread, retrospective evidence of perineural spread, each nerve involved (Trigeminal, V1, V2, palatine, pterygopalatine ganglion, V3, auriculotemporal, meckel’s cave, Facial, chorda tympani, geniculate ganglion, Glossopharyngeal, Vagus, Accessory nerve, and Hypoglossal), extracapsular spread, and skull base. Log-Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests were run to determine if there were differences in survival distribution with each nerve involved and survival differences based on whether PNI and PNS were initially reported on both histopathologic diagnosis and imaging studies.

Histopathologic evidence of perineural invasion:

Using the synoptic report from the initial pathology reports from the total resections, presence or absence of PNI was recorded. From this data, survival analysis showed no significance in distribution between overall and disease-free survival with PNI
with Log Rank test (OS: $C^2=0.033$  $p=0.856$)(DFS: $C^2= 0.003$ $p=0.954$), Breslow (OS: $C^2=0.087$  $p=0.768$)(DFS: $C^2=0.001$  $p=0.981$) and Tarone-Ware(OS: $C^2=0.067$  $p=0.796$)(DFS: $C^2=0.000$  $p=0.998$). Interestingly, the retrospective evidence of PNI was also not statistically significant at the $\alpha = 0.05$ level for overall and disease-free survival although the survival analysis showed more late failures in cases positive for PNI with Log-Rank test (OS: $C^2=2.615$  $p=0.106$)(DFS: $C^2=3.607$  $p=0.058$), Breslow (OS: $C^2=1.671$  $p=0.196$)(DFS: $C^2=2.559$  $p=0.110$) and Tarone-Ware(OS: $C^2=2.094$  $p=0.148$)(DFS: $C^2=3.033$  $p=0.082$). The category “close PNI” defined as positive PNI or close to nerves showed no significant survival distribution differences with Log-Rank test (OS: $C^2=0.692$  $p=0.406$ )(DFS: $C^2=0.893$  $p=0.345$), Breslow (OS: $C^2=0.231$  $p=0.631$)(DFS: $C^2=0.336$  $p=0.562$) and Tarone-Ware(OS: $C^2=0.421$  $p=0.516$)(DFS: $C^2=0.567$  $p=0.451$). With differences in survival noted, a Chi-squared test to determine differences in the initial report of PNI to retrospective evidence of PNI showed that it was reported less often on initial review ($C^2=4.45$  $p=0.035$).
Figure 11. Kaplan Meier plot showing overall survival differences of patients with (green) and without (blue) PNI on initial pathology report.

Figure 12. Kaplan Meier plot showing disease-free survival differences of patients with (green) and without (blue) PNI on initial pathology report.
Figure 13. Kaplan Meier plot showing overall survival differences of patients with (green) and without (blue) PNI on retrospective pathology review.

Figure 14. Kaplan Meier plot showing disease-free survival differences of patients with (green) and without (blue) PNI on retrospective pathology review.
Figure 15. Kaplan Meier plot showing overall survival differences of patients with (green) and without (blue) “Close PNI” on retrospective pathology review.

Figure 16. Kaplan Meier plot showing disease-free survival differences of patients with (green) and without (blue) “Close PNI” on retrospective pathology review.

Trigeminal nerve:

Cranial nerve V (Trigeminal) and several of its branches were evaluated including V1 (ophthalmic), V2 (maxillary), and V3 (mandibular). Meckel’s cave containing the
trigeminal ganglion was also evaluated. Thirty-three (68.6%) of the patients had some Trigeminal nerve involvement. No patients in this study had V1 involvement. Twenty-seven (56.2%) patients had V2 involvement, all 27 (56.2%) involved the palatine nerve and 13 (27.0%) patients had involvement of the pterygopalatine fossa. Thirty-three (68.8%) patients had V3 involvement and 24 (50%) had auriculotemporal invasion. Seven (14.6%) patients had tumor involving the Meckel (trigeminal) cave. The mean overall and disease-free survival for patients with Trigeminal nerve involvement was 53.5 and 44.7 months respectively compared to those without who had a mean survival of 115.5 and 113 months respectively with Log-Rank test (OS: $C^2=10.865\ p=0.001$)(DFS: $C^2=12.389\ p=0.000$), Breslow (OS: $C^2=9.693\ p=0.002$) (DFS: $C^2=11.056\ p=0.001$) and Tarone-Ware (OS: $C^2=10.388\ p=0.001$) (DFS: $C^2=11.863\ p=0.001$). V2 involvement occurred in 56.2% (n=27) of patients with statistically significant survival differences. The mean overall and disease free survival for those with involvement was 58 months and 49 months respectively, compared to 91 and 88 months without V2 invasion with Log-Rank test (OS: $C^2=4.916\ p=0.027$)(DFS: $C^2=5.580\ p=0.018$), Breslow (OS: $C^2=4.690\ p=0.030$)(DFS: $C^2=4.530\ p=0.033$) and Tarone-Ware(OS: $C^2=4.873\ p=0.027$)(DFS: $C^2=5.085\ p=0.024$). The medians were both much lower than the means with OS=16 months and DFS=12 months for V2 invasion, however all cases were censored thus no median for patients without V2 involvement could be calculated. Palatine nerve (branch of V2) involvement was noted in 56.2% (n=27) and also showed statistically significant differences in overall and disease-free survival with Log-Rank test (OS: $C^2=5.026\ p=0.025$)(DFS: $C^2=5.839\ p=0.016$), Breslow (OS: $C^2=4.945\ p=0.026$)(DFS: $C^2=5.264\ p=0.022$) and
Tarone-Ware (OS: $C^2=5.054$ p=0.025)(DFS: $C^2=5.622$ p=0.018). Mandibular (V3) nerve involvement showed dramatically different survival differences with Log-Rank test (OS: $C^2=13.761$ p=0.000)(DFS: $C^2=13.323$ p=0.000), Breslow (OS: $C^2=11.936$ p=0.001)(DFS: $C^2=11.500$ p=0.001) and Tarone-Ware(OS: $C^2=13.008$ p=0.000)(DFS: $C^2=12.587$ p=0.000). Auriculotemporal nerve (branch of V3) did not show a difference in survival. The median overall and disease-free survival for patients with Meckel cave involvement was 12 and 9 months, respectively, compared to 76 months for patients without with Log-Rank test (OS: $C^2=11.304$ p=0.001)(DFS: $C^2=12.195$ p=0.000), Breslow (OS: $C^2=13.371$ p=0.000)(DFS: $C^2=12.484$ p=0.000) and Tarone-Ware(OS: $C^2=12.444$ p=0.000)(DFS: $C^2=12.506$ p=0.000).

Figure 17. Kaplan Meier plot for overall survival of patients with (green) and without (blue) Trigeminal nerve involvement
Figure 18. Kaplan-Meier disease-free survival plot for patients with Trigeminal nerve involvement (green) vs without (blue).

Figure 19 Kaplan-Meier overall survival plot for patients with maxillary division of Trigeminal (V2) nerve involvement (green) vs without (blue).
Figure 20. Kaplan-Meier disease-free survival plot for patients with maxillary division of Trigeminal (V2) nerve involvement (green) vs without (blue).

Figure 21. Kaplan-Meier overall survival plot for patients with palatine branch of Trigeminal (V2) nerve involvement (green) vs without (blue).
Figure 22. Kaplan-Meier disease-free survival plot for patients with palatine branch of Trigeminal (V2) nerve involvement (green) vs without (blue).

Figure 23. Kaplan-Meier overall survival plot for patients with pterygopalatine fossa (V2) nerve involvement (green) vs without (blue).
Figure 24. Kaplan-Meier overall survival plot for patients with pterygopalatine fossa (V2) nerve involvement (green) vs without (blue).

Figure 25. Kaplan-Meier overall survival plot for patients with mandibular division of Trigeminal (V3) nerve involvement (green) vs without (blue).
Figure 26. Kaplan-Meier disease-free survival plot for patients with mandibular division of Trigeminal (V3) nerve involvement (green) vs without (blue).

Figure 27. Kaplan-Meier overall survival plot for patients with auriculotemporal branch of Trigeminal (V3) nerve involvement (green) vs without (blue).
Figure 28. Kaplan-Meier disease-free survival plot for patients with auriculotemporal branch of Trigeminal (V3) nerve involvement (green) vs without (blue).

Figure 29. Kaplan-Meier overall survival plot for patients with trigeminal (Meckel’s) cave involvement (green) vs without (blue).
Facial nerve:

Cranial nerve VII (Facial nerve) and several of its branches were evaluated including chorda tympani, vidian nerve (which contains fibers of the facial via greater petrosal), and the geniculate ganglion. Thirty-seven patients (77.1%) showed some Facial nerve involvement. Mean overall survival for patients with and without CN VII involvement was 62.3 months and 109.5 months respectively with with Log-Rank test (OS: $C^2=4.871$ $p=0.027$), Breslow (OS: $C^2=4.560$ $p=0.033$) and Tarone-Ware(OS: $C^2=4.767$ $p=0.029$). Of note, the median survival for involvement of CN VII was 36 months. Mean disease-free survival for patients with and without CN VII was 55 months and 108 months respectively with Log-Rank test (DFS: $C^2=5.786$ $p=0.016$), Breslow (DFS: $C^2=5.502$ $p=0.019$) and Tarone-Ware (DFS: $C^2=5.721$ $p=0.017$) and the median was 16 months for patients with PNS to CN VII. Thirty-three (68.8%) patients had chorda tympani invasion, 16 (33.3%) had invasion of the Vidian nerve, and 12 (25%)
patients had tumor involving the geniculate ganglion. While chorda tympani involvement did show statistical significance for overall survival only with Log-Rank test (OS: \( C^2= 3.911 \) p=0.048)(DFS: \( C^2=3.316 \) p=0.069), differences early in the survival curve with Breslow (OS: \( C^2=2.600 \) p=0.107)(DFS: \( C^2=1.863 \) p=0.172) and in the middle of the survival curve with Tarone-Ware(OS: \( C^2=3.280 \) p=0.07)(DFS: \( C^2=2.567 \) p=0.109) were not. Overall and disease-free survival distributions for vidian nerve and geniculate ganglion were significant. The mean overall and disease-free survival for vidian nerve involvement was OS=54 months and DFS=47 months compared to OS=79 months and DFS=78 months of those without with Log-Rank test (OS: \( C^2=3.602 \) p=0.058)(DFS: \( C^2=4.503 \) p=0.034), Breslow (OS: \( C^2=5.114 \) p=0.024)(DFS: \( C^2=6.065 \) p=0.014) and Tarone-Ware(OS: \( C^2=4.483 \) p=0.034)(DFS: \( C^2=5.500 \) p=0.019). The median survival however was only 11 months. The mean overall and disease-free survival for geniculate ganglion involvement was OS=36 months and DFS=16 months (median: OS=19 months, DFS=11 months) compared to 133 months (median: OS and DFS=133 months) of those without with Log-Rank test (OS: \( C^2=9.094 \) p=0.003)(DFS: \( C^2=11.892 \) p=0.001), Breslow (OS: \( C^2=8.456 \) p=0.004)(DFS: \( C^2=11.415 \) p=0.001) and Tarone-Ware(OS: \( C^2=8.800 \) p=0.003)(DFS: \( C^2=11.761 \) p=0.001).
Figure 31. Kaplan-Meier overall survival plot for patients with Facial nerve involvement (green) vs without (blue).

Figure 32. Kaplan-Meier disease-free survival plot for patients with Facial nerve involvement (green) vs without (blue).
Figure 33. Kaplan-Meier overall survival plot for patients with chorda tympani branch of Facial nerve involvement (green) vs without (blue).

Figure 34. Kaplan-Meier disease-free survival plot for patients with chorda tympani branch of Facial nerve involvement (green) vs without (blue).
Figure 35. Kaplan-Meier overall survival plot for patients with vidian nerve involvement (green) vs without (blue).

Figure 36. Kaplan-Meier disease-free survival plot for patients with vidian nerve involvement (green) vs without (blue).
Glossopharyngeal nerve

Thirty-seven (77.1%) patients had cranial nerve IX (Glossopharyngeal) involvement. No difference in survival distribution was seen for overall or disease-free
survival with Log-Rank test (OS: $C^2=3.179$ $p=0.075$) (DFS: $C^2=2.477$ $p=0.115$),
Breslow (OS: $C^2=2.737$ $p=0.098$) (DFS: $C^2=1.741$ $p=0.187$) and Tarone-Ware (OS: 
$C^2=3.001$ $p=0.083$) (DFS: $C^2=2.113$ $p=0.146$). However, the mean overall and disease-
free survival was 64 and 61 months (median: OS=34 months, DFS=19 months) compared 
to 97 and 90 months for those without. The 2 year survival for CN IX involvement was 
approximately 55% compared to 90% in those without involvement.

Figure 39. Kaplan-Meier overall survival plot for patients with Glossopharyngeal nerve involvement 
(green) vs without (blue).
Vagus nerve

Of the patients in this study, 31 (64.6%) of patients had Vagus nerve involvement. The mean overall survival was 65 months for those with CN X involvement (median= 55 months) and 87 months for those without. The mean disease-free survival was 61 months regardless of nerve involvement (median= 25 months). Thus, cranial nerve X (Vagus) did not show statistically significant survival distribution differences with Log-Rank test (OS:$C^2=2.234 \ p=0.135$)(DFS:$C^2=1.896 \ p=0.169$), Breslow (OS:$C^2=2.338 \ p=0.126$)(DFS:$C^2=1.949 \ p=0.163$) and Tarone-Ware (OS:$C^2=2.292 \ p=0.130$)(DFS:$C^2=1.929 \ p=0.165$).

Figure 40. Kaplan-Meier disease-free survival plot for patients with Glossopharyngeal nerve involvement (green) vs without (blue).
Figure 41. Kaplan-Meier overall survival plot for patients with Vagus nerve involvement (green) vs without (blue).

Figure 42. Kaplan-Meier disease-free survival plot for patients with Vagus nerve involvement (green) vs without (blue).

Accessory Nerve (CN XI):

Nineteen (39.6%) patients showed evidence of tumor along the Accessory nerve. Median overall and disease-free survival for CN XI PNS was 17 months and 11 months,
respectively, compared to 133 months for patients without. Hence, cranial nerve XI (Accessory) showed decreased survival if involved with Log-Rank test (OS: $C^2=12.853$ $p=0.000$) (DFS: $C^2=14.075$ $p=0.000$), Breslow (OS: $C^2=12.815$ $p=0.000$) (DFS: $C^2=14.381$ $p=0.000$) and Tarone-Ware (OS: $C^2=13.030$ $p=0.000$) (DFS: $C^2=14.475$ $p=0.000$). Involvement of this nerve may manifest as muscle atrophy of the trapezius.

![Survival Functions](image)

Figure 43. Kaplan-Meier overall survival plot for patients with Accessory nerve involvement (green) vs without (blue).
Figure 44. Kaplan-Meier disease-free survival plot for patients with Accessory nerve involvement (green) vs without (blue).

Hypoglossal nerve

Cranial nerve XII invasion was noted in 29 (60.4%) patients. Significant survival distribution differences were observed with the mean of overall and disease-free survival of 54 and 50 months respectively for those with involvement (median: OS=26 months, DFS=16 months) compared to 100 and 95 months of those without with Log-Rank test (OS: $C^2=7.884 \ p=0.005$)(DFS: $C^2=7.453 \ p=0.006$), Breslow (OS: $C^2=6.829 \ p=0.009$)(DFS: $C^2=6.2999 \ p=0.012$)and Tarone-Ware(OS: $C^2=7.414 \ p=0.006$)(DFS: $C^2=6.927 \ p=0.008$).
Other prognostic factors evaluated were radiographic evidence of extracapsular spread and skull base involvement. Extracapsular invasion noted on radiographs did not show statistically different survival distribution with Log-Rank test (OS:}
C^2=2.018  p=0.155)(DFS: C^2=1.665  p=0.197 ), Breslow (OS: C^2=2.386 p=0.167)(DFS: 
C^2=1.912  p=0.167)and Tarone-Ware(OS: C^2=2.209 p=0.137)(DFS: C^2= 
1.792  p=0.181). The median overall survival for patients with skull base involvement 
was 19 months compared to 133 months for those without. The median disease-free 
survival was 11 months compared to 133 months. Thus, survival distribution differences 
were noted with Log-Rank test (OS: C^2=12.623 p=0.000)(DFS: C^2=14.567  p=0.000), 
Breslow (OS: C^2=12.451 p=0.000)(DFS: C^2=15.640  p=0.000)and Tarone-Ware(OS: 
C^2=12.608 p=0.137)(DFS: C^2= 15.303  p=0.000).

![Figure 47. Kaplan Meier plot showing overall survival of patients with (green) and without (blue) extracapsular invasion.](image-url)
Figure 48. Kaplan Meier plot showing disease-free survival of patients with (green) and without (blue) extracapsular invasion.

Figure 49. Kaplan Meier plot of overall survival of patients with skull base involvement (green) vs without (blue).
Figure 50. Kaplan Meier plot of overall survival of patients with skull base involvement (green) vs without (blue).
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Table 1: Overall survival by nerve involvement
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Table 2: Disease-free survival by nerve involvement
Anaplasia

Each case (n=193) was examined for anaplasia. Survival distributions (Kaplan Meier), relationship with smoking (Pearson’s Chi-squared test), and relationship to PNI (Pearson’s Chi-squared test) were examined. Contrary to previous reports\textsuperscript{15,16} our data did not show a decrease in survival of patients with anaplasia with Log-Rank test ($\chi^2=0.791$  $p=0.3737$) or Wilcoxon test ($\chi^2=0.785$  $p=0.3756$). Interestingly, anaplasia showed no relationship to smoking ($\chi^2=0.243$  $p=0.622$), regardless of pack-year history (2-tailed t test $p=0.477$), current, former, or never status ($\chi^2= 1.422$  $p=0.700$)

Figure 51. Kaplan-Meier survival plot of anaplasia (blue) vs no anaplasia (red)
Chapter 4: Discussion and Conclusion

Many factors must be evaluated to determine a patient’s prognosis and best treatment for a positive outcome. This study focused on advanced stage cancer patients and sought to determine attributes associated with failures (death or recurrence) and better predictors of these events.

The absence of statistical significance in overall survival regardless of initial reported evidence of histopathologic or imaging neural involvement could suggest several things. It could represent, as hypothesized by the authors, that neural involvement is consistently under called, both microscopically and by imaging, and the patients are experiencing failures due to lack of proper treatment. Another possibility is that these patients are dying of other disease-related problems regardless of neural invasion status. While both of these are confounding contributions to the failure of advanced stage SCC patients, more thorough and accurate diagnosis should provide a better chance for sufficient treatment to control disease. Disease-free survival was also not statistically significantly different for patients with and without histopathologic initial report of PNI. Interestingly, disease-free survival for the initial radiology reported evidence of PNS was statistically significant. A possible explanation for this would be that patients with advanced cancers have obvious PNS and die more quickly, while patients with early cancers have more subtle PNS and may fail much later. Regardless of the statistical significance, 60% of patients without evidence on initial report of imaging
of PNS were dead of disease at 2 years. This could suggest that PNS has not been recognized, or that patients are dying of other disease sequelae.

In an effort to determine if neural invasion was overlooked or if other factors alone resulted in the failure of these patients, the authors compared the retrospective findings to the initial findings. As expected, the retrospective evaluation found nerve involvement significantly more often than the initial review of both pathology and imaging. Possible explanations of this include the vague and open-to-interpretation definition of PNI on pathology, significant caseload, and relative inexperience of the pathologist.

Distributions in overall and disease-free survival for retrospective evidence of histopathologic PNI was not statistically significant although, as seen in (Figure X-KM chart of retro PNI), many late failures occurred. This could be a result of tumor moving slowly along nerves away from the site of treatment, failures due to other disease-related complications or, most likely, both. Retrospective radiographic evaluation of PNS in this cohort was somewhat alarming in that all the patients without PNS survived, whereas those with PNS had an overall two-year survival of less than 60% and a disease-free survival of less than 50% at 2 years.

In an evaluation of six cranial nerves (V, VII, IX, X, XI, XII), survival distributions were statistically significant for CN V (Trigeminal), CN VII (Facial), CN IX (Accessory), and CN XII (Hypoglossal). CN IX (Glossopharyngeal) and X (Vagus) involvement did not affect survival outcomes.

CN V: Trigeminal nerve was viewed entirely from trigeminal cave to its three divisions (ophthalmic, maxillary, and mandibular) inferiorly to smaller branches
including V2 branches in the pterygopalatine fossa and palatine and V3 branches auriculotemporal, lingual, and inferior alveolar. No patients had ophthalmic division (V1) involvement. Interestingly, both V2 and V3 involvement had impressive survival distribution differences compared to patients without (See KM chart V2 and V3). The two-year survival for V2 involvement less than 60% while those without had a survival of eighty percent in the same time period. While the palatine nerve involvement did affect patient outcome, tumor in the pterygopalatine fossa did not show statistically significant differences in overall survival. A possible explanation for this could be that the pterygopalatine fossa is a larger space therefore more tumor with effacement of fat must be present for observable tumor involvement. This could result in under-calling tumor involvement in this space. Interestingly, while the Log-Rank test for overall survival was not significant, Breslow and Tarone-Ware both showed significance suggesting differences early and in the middle of the curve, respectively. Unsurprisingly, Meckel’s cave involvement had a dramatically reduced survival, an expected result once tumor enters the skull. The mandibular division (V3) of the Trigeminal nerve had arguably the most significant survival distribution in that all patients without V3 involvement lived (censored data), with two-year survival of less than fifty percent and five-year survival of less than thirty percent in patients positive for perineural spread along this nerve.

CN VII: Facial nerve was viewed entirely from the geniculate ganglion through its course inferiorly including its branch chorda tympani. Survival distribution for chorda tympani involvement was not significant however the facial nerve and geniculate ganglion involvement did show worse prognosis with a five-year survival of less than
35% and less than 20%, respectively. Facial nerve also gives off the branch greater petrosal that courses through the pterygopalatine fossa where it joins with sympathetic fibers from the carotid plexus (deep petrosal nerve) to form the vidian nerve. Vidian nerve involvement was statistically significant for early differences (Breslow) and middle differences (Tarone-Ware) with a significantly decreased survival with a 5 year survival of 35% (compared to over 60% without). However, Log-Rank test was not statistically significant with a p value slightly above the significance value.

CN IX, X, XI: Glossopharyngeal, Vagus, and Accessory nerve all exit the skull through the jugular foramen. Tumor spread through the neck can affect any, all, or none of these nerves. While perineural spread of Glossopharyngeal and Vagus did not show statistically significant decreased overall or disease-free survival, Accessory nerve involvement did affect prognosis. Accessory (Spinal Accessory) nerve involvement could be observed in patients with denervation atrophy of the trapezius, a muscle innervated by this nerve. (See Figure 52) Survival distribution was significantly different for patients with Accessory nerve involvement (2 year survival of 40%, 5 year survival of 20%).
Figure 52: Axial T2 MRI depicting tumor (yellow arrow) on the left side with denervation atrophy of the trapezius muscle (red arrows).

CN XII: Hypoglossal nerve exits the skulls through the hypoglossal canal traveling inferior and medial to innervate genioglossus, hyoglossus, styloglossus, and intrinsic muscles the tongue. Hypoglossal perineural spread can manifest radiographically as fatty denervation atrophy or enlarged nerve and clinically as deviation toward the affected side upon protrusion.

Anaplasia:
Anaplasia has been cited in several studies as a prognostic factor resulting in decreased overall and disease-free survival. Interestingly, using Lewis et al. criteria for anaplasia, the authors found no difference in survival distribution of patients regardless of anaplasia. The chi-squared analysis showed no relationship between smoking and anaplasia, regardless of current, recent-former, remote-former, or never smoker. Anaplasia also showed no relationship with perineural invasion suggesting it is a different mechanism entirely. As expected, perineural invasion (on the retrospective evaluation) did negatively affect prognosis. While numerous factors can affect the outcome in head and neck cancers, the mechanisms of anaplasia and perineural invasion appear to be different and unrelated.

Conclusion

Cranial nerve involvement of tumor results in higher likelihood recurrence and greater morbidity. Specifically, involvement of the Trigeminal nerve (V2 and V3), including Meckel’s (trigeminal cave), Facial nerve along the major branches and as the nerve approached the skull (vidian and geniculate ganglion), Accessory nerve and Hypoglossal nerve showed decreased disease-free and overall survival. Identification of perineural spread would potentially improve complete treatment of the tumoral extension by either expanding the field of radiation or surgery and maximizing the chance for an optimal outcome.

In this study anaplasia did not impact survival or have a significant association with tobacco exposure or perineural invasion. Although PNI which is a histopathologic diagnosis looking for greater than 33% encirclement of nerves by tumors did not
correlate with PNS, the presence of PNS along the Facial, Trigeminal, Spinal Accessory and Hypoglossal nerves were associated with a significant reduction in disease free and overall survival. In order to improve patient outcomes, the extent of both the primary tumor and the presence of PNS must be adequately determined and treated at the outset, requiring an awareness of the potential perineural pathways. The radiographic features of PNS and the full extent of the PNS, are imperative findings that must be carefully assessed to ensure proper treatment for head and neck cancer patients and reduce morbidity and mortality.

Study limitations

Due to a small number of data (n=48), this study has low power. In addition to the small numbers, disease-free survival was hard to assess due to electronic records, patient compliance and patients choosing other care facilities for adjuvant therapy. This study also focused on advanced-stage cancers, thus the patients were more likely to have a greater number of imaging studies available for analysis. In addition, the years of this study precluded proper assessment of HPV status without further tests. While some tumors appear to have a propensity for nerves, the authors did not investigate the possible genetic component allowing these tumors to track along the nerves both in an anterograde and retrograde fashion.
Areas for future research

Future studies with a larger sample size, including earlier stages of cancer would be beneficial to determine the prognostic significance of early diagnosis of perineural spread on MRI. Comparing head and neck radiology to neuroradiology reports could reveal areas for improvement in rendering consistent and accurate assessment of disease extent, that could include enhanced curricular emphasis within radiologic training programs. In addition, analysis of the genetic makeup of tumors with and without perineural invasion could lead to targeted therapies for otherwise relentless disease.
References


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Table 3. Patient demographics for imaging review
Appendix B: Data
Table 4. Initial radiology PNS call vs retrospective PNS call

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Table 6. Anaplasia vs Smoking (0/1), no statistically-significant difference

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Table 7. Anaplasia by Smoking status, no statistically-significant difference
Appendix C: Perineural invasion photomicrographs
Figure 53. Representative photomicrograph of PNI H&E 20X.

Figure 54. High power view of PNI H&E 40X
Figure 55. Tumor within the nerve sheath. 20X

Figure 56. Tumor encircling nerve more than 270 degrees.
Figure 57. Squamous cell carcinoma in close proximity to and within nerve fiber 20X

Figure 58. Low power representative photomicrograph of tumor within nerve sheath.
Figure 59. Medium power photomicrograph showing nerve in close proximity to tumor but not fitting the criteria as PNI.

Figure 60. Small nerve encircled by tumor.
Figure 61. Two small nerves shown, with tumor surrounding the nerve by greater than 180 degrees.

Figure 62. Tumor cells completely surround a nerve. Medium power H&E.
Figure 63. Tumor surrounding greater than 33% of the nerve.

Figure 64. Medium power view of a nerve in close proximity to tumor, not fitting the criteria for PNI
Figure 65. Low power photomicrograph of tumor exhibiting PNI.

Figure 66. Representative photomicrograph of PNI.
Figure 67. Tumor cells surrounding more than half of a small nerve.

Figure 68. Small nerve fiber surrounded by tumor cells.
Figure 69. Tumor cells encircling a nerve bundle.

Figure 70. Multiple nerves in close proximity to and surrounded by malignant cells.
Figure 71. Large nerve fiber with tumor.

Figure 72. Tumor (top of image) exhibiting necrosis and invasion into nerve fiber.
Figure 73. Squamous cell carcinoma tracking along a large nerve fiber.

Figure 74. Tumor encircling at least 33% of nerve.
Figure 75. Tumor surrounding nerve 20X

Figure 76. Tumor within a large nerve. 10X
Appendix D: Anaplasia photomicrographs
Figure 77. Representative photomicrograph showing anaplasia 40X

Figure 78. Representative photomicrograph showing numerous cells at least 5 times the size of a lymphocyte.
Figure 79. Representative photomicrograph showing anaplasia

Figure 80. Representative photomicrograph showing anaplasia 40X
Figure 81. Malignant cells exhibiting increased nuclear size. 40X

Figure 82. Nest of tumor cells exhibiting necrosis with more than three anaplastic cells in this 40X field.
Appendix E: Representative imaging results
Figure 83. Axial T1 MRI exhibiting asymmetric fullness in the right tonsil with fatty denervation of the right oral tongue.

Figure 84. Axial T2 MRI exhibiting asymmetric fullness in the right tonsil with fatty denervation of the right oral tongue (same patient as figure 72).
Figure 85. Coronal MRI showing tumor in the right tonsil (same patient as Figures 72 and 73)

Figure 86. Saggital view of right tonsillar cancer (same patient as 72-74)