

**THE UNIVERSITY OF VIRGINIA  
DEPARTMENT OF OTOLARYNGOLOGY – HEAD & NECK SURGERY  
PRESENTS**

**THE 45th ANNUAL FITZ-HUGH SYMPOSIUM**

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**RESIDENT RESEARCH PRESENTATIONS**

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**Friday June 18, 2021 at 8:30 am**

**Pinn Hall**

**Basic/Translational/Applied Science**

- Using CRISPR to create an HA-tagged knock-in mouse to localize ATP8B1  
in the hair cell's stereocilia subdomains ..... Jonas (Shin)
- Novel in vivo leporine phonation model for glottic insufficiency ..... Churnin (Daniero)
- Effects of emu oil and statins on sinonasal symptoms and quality of life in  
patients with refractory chronic rhinosinusitis—research block update ..... Basu (Mattos)
- Bell's palsy: seeking the answers in social media ..... Iorio (Oyer)
- Machine learning and radiographic biomarkers of head and neck cancer,  
a collaborative approach ..... Mella (Garneau)
- An unsupervised analysis of type 1 laryngeal cleft phenotypes and outcomes in  
children who undergo diagnostic laryngoscopy for severe respiratory symptoms ..... Saez (Early/Brand)

*[break]*

**Clinical Science**

- Utility and outcomes of circumferential peripheral margins in  
head and neck melanoma excision ..... Jung (Jameson)
- The effect of vestibular schwannoma growth rate on audiometric performance ..... Casale (Kesser)
- Surgeon review of 4D CT ..... Barker (Shonka)
- Predictors of laryngeal stenosis development and tracheostomy dependence  
after acute laryngeal injury ..... Carpenter (McGarey)
- Predictors of false negative sentinel lymph node biopsy in clinically  
localized Merkel cell carcinoma ..... Bartels (Jameson)

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## RESIDENT RESEARCH ABSTRACTS

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### Using CRISPR to Create an HA-Tagged Knock-In Mouse to Localize ATP8B1 in the Hair Cell's Stereocilia Subdomains

Rachel Jonas MD; Jung-Bum Shin PhD

**Objective:** Use CRISPR to generate a mouse line in which an HA-tag is knocked into the endogenous *Atp8b1* locus (HA-tag at the N-terminus) to localize ATP8B1 in the hair cell's stereocilia subdomains using confocal microscopy.

**Design:** Animal research study

**Methods:** For generation of HA-ATP8B1-N mice we used the online tool CRISPOR to select suitable target sequences. Neonatal mouse pups were sacrificed by rapid decapitation. Genotyping was performed by PCR amplification of the region of interest. For immunofluorescence, the organs were fixed and rabbit anti-HA antibody was used followed by imaging with a scanning confocal microscope. Western blot was performed with both mouse anti-HA antibody and mouse anti-ATP8B1 antibody using the liver from HA-ATP8B1-N mice and wildtype mice. For generation of HA-ATP8B1-C mice we used the online tool CRISPOR to select suitable target sequences.

**Results:** An HA-ATP8B1-N mouse was successfully generated via CRISPR, with normal ABR of a founder mouse. However, we were unable to localize the protein using confocal microscopy. A Western blot was performed, indicating that the HA tag had been cleaved from the ATP8B1 protein, which resulted in loss of immunoreactivity. Therefore, CRISPR was used to knock-in an HA tag to the C-terminus of the *Atp8b1* locus.

**Conclusions:** We have yet to localize ATP8B1 in the hair cell's stereocilia subdomains due to cleavage of the tagging peptide, but are hopeful that addition of the HA tag to the C-terminus will allow for localization.

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### Novel in Vivo Leporine Phonation Model for Glottic Insufficiency

Ian Churnin MD; William Swift MD; James Daniero MD

**Objective:** To develop a novel in-vivo model for glottic insufficiency by simulating reduced unilateral vocal fold mobility.

**Design:** Non-randomized controlled before-after trial. Rabbit subjects underwent single operation of forced phonation with bilateral and unilateral cricothyroid muscle stimulation to assess for effect of stimulation method on glottic cycle and audiometric properties.

**Participants:** Seven female New Zealand white rabbits. One rabbit developed intraoperative laryngeal hematoma leading to exclusion from analysis.

**Interventions:** Bilateral (Control) vs unilateral (Case) cricothyroid muscle stimulation during phonation. Each rabbit underwent both interventions, serving as own control.

**Main Outcome Measures:** Mean Maximum Glottic Area/Length (MGA/Length), Mean Right Amplitude/Length, Mean Left Amplitude/Length, Mean Frequency [Hz], Mean Maximum Intensity [dB]

**Results:** Mean MGA/length was significantly greater with unilateral vs bilateral stimulation (20.3 vs 9.62,  $p=0.043$ ). No significant difference of mean right amplitude/length between bilateral and unilateral stimulation. Mean left amplitude/length trended towards significance between bilateral vs unilateral stimulation (0.11 vs 0.06,  $p=0.059$ ). Mean frequency was significantly reduced with unilateral vs bilateral stimulation (479.9Hz vs 683.5Hz,  $p=0.027$ ). Mean maximum intensity was significantly reduced with unilateral vs bilateral stimulation (76.3dB vs 83.5dB,  $p=0.013$ ).

**Conclusions:** Data analysis comparing glottic gap, pitch frequency and sound intensity revealed expected significant differences between bilateral and unilateral stimulation consistent with reduced unilateral vocal fold mobility glottic insufficiency supporting this methodology to be utilized as a novel single surgery in-vivo leporine phonation model for glottic insufficiency.

## **Effects of Emu Oil and Statins on Sinonasal Symptoms and Quality of Life in Patients with Refractory Chronic Rhinosinusitis—Research Block Update**

**Annesha Basu MD MS**; Lydia Weykamp MD; Andrew Strumpf MPH; Jose Mattos MD MPH

**Objective:** Study the role of novel therapies in patients with chronic rhinosinusitis refractory to standard topical and systemic medical treatment.

**Design/Setting:** Clinical Trial

**Setting:** Academic tertiary care hospital, Private Practice clinic

**Patients:** Our goal is to recruit 84 patients total; this sample size was calculated based on a prior study which had recommended a minimal clinically important difference (MCID) of 12 for SNOT-22 scores of CRS patients undergoing medical management.

**Interventions(s):** For the statin study, patients will be randomized with a 1:1 ratio between the Rosuvastatin intervention arm and placebo arm.

For the emu oil study, our intervention will require participants to administer three to four drops of emu oil twice daily to each nostril using a dropper, with the patient in the Mygind position

**Main Outcome Measures:** Determine whether statin medications improve objective metrics of disease and disease-specific quality of life (QOL) in CRS.

**Results:** For the statin aspect of the study, a CORE grant was submitted January 2021 for the American Rhinologic Society Resident Research Grant.

For the emu oil study, an Investigational New Drug (IND) application was submitted to the Food and Drug Administration.

**Conclusions:** We are currently awaiting notification of the outcome of the CORE grant application for the statin study.

For the emu oil study, the FDA responded to the IND application and requested additional studies be performed prior to human application.

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## **Bell's Palsy: Seeking the Answers in Social Media**

**Caitlin Iorio MD**; Lydia Weykamp MD; Samuel Oyer MD

**Background:** Forty-two percent of Americans search for health-related information on social media platforms, and forty-five percent of consumers report that social media health information influences their decision to seek care.

**Learning Objectives:** We aimed to characterize the content, sources and overall trends of Bell's Palsy posts on Twitter.

**Study Objective:** Our intent was to analyze Twitter trends surrounding Bell's Palsy and compare healthcare-based tweets to that of the general public.

**Methods:** We utilized Symplur, a healthcare social media analytics company to create a dataset of tweets including "Bell's Palsy" over a three-year period. We excluded spam and non-English speaking accounts.

**Results:** From January 2018 through January 2021, there were 16,178 tweets relating to Bell's Palsy. 1,466 were healthcare-based which had more attached links and media but less replies when compared to the general public which had 14,712 tweets. There was a triple-fold increase in tweets in 2020 amongst the general public when compared to the prior two years. This was largely driven by the Covid19 epidemic. The most common words in the general public and healthcare tweets over three years included vaccine, Pfizer and paralysis. One of the top three hashtags for the general public was "#Covid19" while this was not in the top three for healthcare-based tweets.

**Conclusion:** There is a trend of increasing tweets among the general public about Bell's Palsy in 2020 which could be attributed to Covid19 despite no causal relationship between the coronavirus and Bell's palsy.

## Machine Learning and Radiographic Biomarkers of Head and Neck Cancer, a Collaborative Approach

Jeffrey Mella MD; Tom Fletcher PhD; Yinzhu Jin, MSc; Jonathan Garneau MD

**Objective:** Utilize deep neural networks to identify radiographic biomarkers that predict clinically useful features (HPV status, chance of distant metastasis, and extranodal extension) on pretreatment CT images of patients with oropharyngeal carcinoma.

**Methods:** Data from The Cancer Imaging Archive (TCIA) was used to develop a deep neural network model based on radiomics (aspect of machine learning that focuses on pixel interactions and relationships) to predict clinically relevant features (HPV status, ENE, Risk of developing distant metastasis) of OPSCC tumors on CT images. We created a comprehensive model that incorporates raw features CT images (tumor size, aspect ratio) to evaluate chance of distant metastasis as preliminary validation of our model.

**Results:** 462 pts with OPSCC were divided into training (N=180) and validation cohorts (N = 282). Radiomics parameters and ROC curves were designed to evaluate the prediction of HPV status, ROC curves for training (AUC = 0.84) and validation (AUC = 0.7) denoting a strong classifier. 298 pts with OPSCC were then divided into training (N= 199) and validation cohorts (N= 99). “Alignment scores” were calculated evaluating both “aspect ratio” and “tumor size” demonstrating statistical significance (p-value <0.001).

**Conclusion:** Machine learning has demonstrated the ability to identify radiographic biomarkers (objective, quantitative parameters that are reflective of an oropharyngeal tumor’s molecular signature). Based on successful delineation on preliminary data, the machine learning algorithm will be applied to further clinically useful data points such as distant metastasis and ENE. Our future prospective study will be designed to create a UVA database for further generalizability of deep neural networks.

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## An Unsupervised Analysis of Type 1 Laryngeal Cleft Phenotypes and Outcomes in Children Who Undergo Diagnostic Laryngoscopy for Severe Respiratory Symptoms

Neil Saez MD; Xizhao Chen MS4; Sebastian Dobrow MS4; William Teague MD; Stephen Early MD; William Brand MD

**Objectives:** To identify meaningful type 1 laryngeal cleft (LC1) phenotypes through unsupervised hierarchical cluster analysis.

**Setting and Patients:** All children who underwent laryngoscopy for severe respiratory or swallowing symptoms and were diagnosed with an LC1 in a single tertiary care center over a 7 year period.

**Methods:** Variables indicating pre-diagnosis respiratory and swallowing signs and symptoms, health care utilization, structural anomalies, inflammation markers, and recovered bronchoalveolar lavage (BAL) components were reduced with factor analysis, categorized by cluster analysis, validated by discriminant function analysis, and confirmed in multiple replication samples. Prospectively-collected patient-reported outcome measures and ultimate need for surgical repair were analyzed between clusters.

**Results:** The analysis was repeatable and accurately categorized 85.3 % of cases. Cluster 1 (n=83) had increased BMI, higher prevalence of lipid-laden macrophages, larger tonsil size, and symptoms of laryngeal dysfunction. Cluster 2 (n=43) were younger, more likely to have symptoms of dysphagia and bronchial hyper-reactivity, less likely to have severe comorbidities, and more likely to tolerate thin liquids. Cluster 3 (n=40) was older at time of symptom onset and diagnosis, had increased preoperative health care utilization, with highest prevalence of pneumonias and eosinophilic esophagitis and asthma. Cluster 4 (n=18) had the highest rates of bronchopulmonary dysplasia, neurologic comorbidities, parental nutrition, BAL lymphocytosis, elevated BAL cell count and CRP. Cluster 1 had a trend towards increased need for surgical repair (Chi-square=5.374, p=0.148).

**Conclusions:** Cluster analysis offers a novel multidimensional approach for identifying LC1 phenotypes that exhibit differences in need for surgical repair.

## Utility and Outcomes of Circumferential Peripheral Margins in Head and Neck Melanoma Excision

Hyunseo Jung MD; Michael McWilliams; Mark Jameson MD PhD

**Objective:** To assess the utility of reduced margins in conjunction with peripheral margin pathologic analysis in excision of head and neck melanoma by comparing the rate of re-excision, recurrence and long-term survival compared to patients treated according to guideline-recommended wide excision margins.

**Design:** Retrospective cohort study

**Setting:** Single center, single provider study at UVA health system

**Patients:** 132 adult patients who underwent excision of cutaneous melanoma of head and neck with Dr. Jameson within the past 3 years.

**Intervention:** Reduced margin width with circumferential peripheral en face margin assessment.

**Main outcomes:** Average margin width, rate of re-excision, rate of locoregional recurrence (LRR), disease-free survival (DFS), disease-specific survival (DSS), overall survival (OS).

**Results:** The mean margin width was consistently lower than recommended guidelines among our patients with the exception of stage 0 (Tis) lesions. Rate of negative en face margins remained 90% across all patients. Mean duration of follow up of 11 months. Only two patients (1.5%) experienced LRR during their follow-up period. DFS, DSS and OS were similar to published series suggesting no negative impact this resection technique. However, the number of events within the population was limited.

**Conclusions:** Our method of enhanced margin assessment does not compromise oncologic outcomes from head and neck cutaneous melanoma. This approach likely has advantages including improved cosmetic and/or functional outcomes which warrant further study.

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## The Effect of Vestibular Schwannoma Growth Rate on Audiometric Performance

Garrett Casale MD; Sergio Ferrante MD; Ariel Finberg MD; Bradley Kesser MD

**Objective:** To evaluate the relationship between growth rate of observed vestibular schwannomas and the rate of change in audiometric parameters.

**Study Design:** Retrospective chart review

**Setting:** Tertiary academic medical center

**Patients:** All patients with a diagnosis of vestibular schwannoma evaluated between the years 2004-2019 with full imaging and audiometric data.

**Interventions:** Tumor Observation

**Main Outcome Measures:** Tumor growth rate, pure tone average (PTA), speech reception threshold (SRT), and word recognition score (WRS).

**Results:** Sequential surveillance data from patient audiograms were used to calculate rate of change for PTA, SRT, and WRS for each patient (n=48 patients). Similarly, surveillance MRI data was used to calculate rate of change in tumor volume (average 8.8 mm<sup>3</sup>/month, range -35.2 to 131.2 mm<sup>3</sup>/month). Average follow up time was 899 days (range 148-3493 days). Multivariate linear regression analysis was used to evaluate correlations. For intracanalicular tumors, there was a statistically significant (p=0.002) and highly linear (Pearson correlation coefficient = 0.786) relationship between tumor growth rate and change in PTA. For large tumors with significant CPA extension, there was no correlation observed. There was no statistical correlation between tumor growth rate and change in SRT or WRS.

**Conclusions:** Rate of tumor growth and rate of change of patient PTA appear to have a highly linear relationship for intracanalicular tumors. This relationship is lost once the tumor becomes large with significant extension into the CPA. These data support the conclusion that the effects of tumor compression on the acoustic nerve may play an important role in audiometric decline.

## Surgeon Review of 4D CT

**Simone Barker MD**; Zoe Roecker MS4; Andrew Strumpf MPH; David Shonka MD

**Objective:** To compare the accuracy of surgeon reviewed 4D computed tomography (4D CT) versus radiologist reviewed 4D CT for localization of parathyroid adenomas in patients with primary hyperparathyroidism.

**Design:** A database of patients with hyperparathyroidism who underwent parathyroidectomy at UVA was developed in REDCap. Patients undergoing parathyroidectomy between 1/1/2017 to 1/1/2021 were specifically evaluated. Data collection includes demographics, preoperative evaluation, intraoperative evaluation, and postoperative evaluation.

**Setting:** Tertiary care center

**Main Outcome measures:** sensitivity, specificity, positive predictive value, and negative predictive value for surgeon and radiologist reviewed 4D CT.

**Results:** 20 of patients with primary hyperparathyroidism who had preoperative 4D CT scan imaging available for review were evaluated. Surgeon demonstrated a PPV of 0.93 for localization of parathyroid adenomas on the right sided compared to radiologist who demonstrated a PPV of 0.79 in the setting a prevalence of 0.50. For left sided parathyroid adenoma localization surgeon demonstrated a PPV of 0.70 while radiologists demonstrated a PPV of 0.60 in the setting a prevalence of 0.50.

**Conclusion:** Surgeon localization of parathyroid adenoma on 4D CT scan was more accurate than radiologist localization.

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## Predictors of Laryngeal Stenosis Development and Tracheostomy Dependence After Acute Laryngeal Injury

**Delaney Carpenter MD**; Jessica Lin MS1; Karlie Sivetz; Patrick McGarey MD

**Objectives:** To characterize risk factors for the development of posterior glottic stenosis (PGS) or subglottic stenosis (SGS) and tracheostomy dependence after acute laryngeal injury (ALGI) secondary to intubation-related trauma.

**Study design:** Retrospective chart review

**Methods:** A chart review was performed of all adult patients who underwent tracheostomy for prolonged intubation from 2015-2021. Inclusion criteria included evaluation by a laryngologist within 2 weeks of the initial injury with follow-up at least 8 weeks later. Exclusion criteria included pre-existing airway stenosis, head and neck malignancy, and blunt neck trauma. Primary outcomes were the development of PGS and SGS and tracheostomy presence at 2 and 4 months postoperatively.

**Results:** 823 patients were assessed for eligibility and data from 13 patients with mucosal ALGI were ultimately examined. 6 patients (46%) developed PGS and 9 patients (69%) developed SGS after their injury. 10 patients (77%) remained tracheostomy dependent at 2 months postoperatively, and 7 patients (54%) remained tracheostomy dependent at 4 months postoperatively. The development of PGS was found to be statistically significantly related to patient weight, BMI, and a history of OSA or diabetes ( $P < 0.05$ ). There were no statistically significant relationships between demographic/clinical factors and the development of SGS or tracheostomy dependence at 2 and 4 months postoperatively.

**Conclusions:** PGS development was found to correlate with elevated weight/BMI and OSA or diabetes history, which is supported by existing literature. This study was limited by a small sample size. A prospective multi-institutional study with standardization in data collection would help shed much greater light on this topic.

## **Predictors of False Negative Sentinel Lymph Node Biopsy in Clinically Localized Merkel Cell Carcinoma**

**Harrison Bartels MD**; Richard J Straker III MD; Kevin Lynch MD; Craig Slingluff MD; Mark Jameson MD PhD; *et al.*

**Background:** Sentinel lymph node biopsy (SLNB) is routinely recommended for clinically localized Merkel cell carcinoma (MCC); however, predictors of false negative (FN) SLNB are undefined.

**Methods:** Patients from six centers undergoing wide excision and SLNB for stage I/II MCC (2005–2020) were identified and were classified as having either a true positive (TP), true negative (TN) or FN SLNB. Predictors of FN SLNB were identified and survival outcomes were estimated.

**Results:** Of 525 patients, 28 (5.4%), 329 (62.7%), and 168 (32%) were classified as FN, TN, and TP, respectively, giving an FN rate of 14.3% and negative predictive value of 92.2% for SLNB. Median follow-up for SLNB-negative patients was 27 months, and median time to nodal recurrence for FN patients was 7 months. Male sex (hazard ratio [HR] 3.15,  $p = 0.034$ ) and lymphovascular invasion (LVI) (HR 2.22,  $p = 0.048$ ) significantly correlated with FN, and increasing age trended toward significance (HR 1.04,  $p = 0.067$ ). The 3-year regional nodal recurrence-free survival for males  $>75$  years with LVI was 78.5% versus 97.4% for females  $\leq 75$  years without LVI ( $p = 0.009$ ). Five-year disease-specific survival and overall survival were significantly worse for FN patients.

**Conclusion:** Failure to detect regional nodal microscopic disease by SLNB is associated with worse survival in clinically localized MCC. Males, patients  $>75$  years, and those with LVI may be at increased risk for FN SLNB. Consideration of increased nodal surveillance following negative SLNB in these high-risk patients may aid in early identification of regional nodal recurrences.