Efficacy of CD1a in distinguishing calcifying epithelial odontogenic tumor from amyloid-rich central odontogenic fibroma

Monday, 11th April - 08:00: - Oral (Student/Resident)

Dr. Carter Bruett (New York Presbyterian Queens Hospital), Dr. Spencer Roark (New York Presbyterian Queens Hospital), Dr. Renee Reich (New York Presbyterian Queens), Dr. Paul Freedman (New York Presbyterian Queens)

Introduction: Several reports have suggested that Calcifying Epithelial Odontogenic Tumors (CEOT) are either neg- ative or only focally positive with CD1a. This contention lent support to the notion that CD1a staining would be useful in the diagnosis of a variant of central odontogenic fibromas with amyloid deposits (Amyloid Rich Central Odontogenic Fibroma (AR-COdF)) which are devoid of calcifications and stain strongly with CD1a.

We undertook this pilot study to evaluate CD1a staining in CEOTs and several COdFs to determine CD1a's utility in establishing a diagnosis.

Materials and Methods: As part of this pilot study, 9 CEOTs and 8 COdFs were stained with CD1a. Since CD1a stains Langerhans cells, staining was only expected in association with odontogenic epithelium. Only staining in areas of epithelium was considered a positive result. The staining was graded as strongly positive, focally positive or nega- tive. The CEOTs chosen were diagnosed as classic (n=4), in association with a dentigerous cyst (n=3) and combined CEOT/AOT (n=2). COdF were simple type (n=3), WHO type (n=1), hyalinized with amyloid (n=1) and without (n=1) amyloid, and not-otherwise-specified (n=2). One case of a dentigerous cyst with diffuse amyloid deposition and scattered odontogenic rests was included for comparison.

Results: 7 CEOTs were found to stain positively with CD1a: 5 diffusely, 2 focally. Notably two classic CEOTs without calcifications were strongly positive. The 3 simple-type COdF were negative. Of the 5 remaining, 2 were positive, including the hyalinized-with-amyloid COdF diffusely, the COdF, NOS focally. The amyloid rich cyst was negative. The high percentage of CEOTs that stain strongly with CD1a suggests that CD1a is a poor differentiator between COdF and CEOT.

Conclusion: CD1a is of little value in differentiating odontogenic lesions from each other. Histopathology should remain the primary method for differentiation of COdF from CEOT.

GROWTH CHARACTERISTICS OF AMELOBLASTOMAS AND KERATOCYSTIC ODONTOGENIC TUMORS: A COMPARISON OF 76 TUMORS

Monday, 11th April - 08:12: - Oral (Student/Resident)

Dr. Grayson Cole (University of Pittsburgh), Dr. Suvendra Vijayan (University of Pittsburgh), Dr. Anitha Potluri (University of Pittsburgh), Dr. Elizabeth Ann Bilodeau (University of Pittsburgh)

INTRODUCTION: Ameloblastomas and keratocystic odontogenic tumors (KCOT) are benign odontogenic tumors with overlapping clinical and radiographic presentations. KCOTs are reported to proliferate within cancellous bone without cortical expansion, whereas ameloblastomas expand the cortices at a higher rate. This can be a helpful tool when developing a clinical differential diagnosis. However, this hypothesis has not been validated through three-dimensional imaging techniques with a large cohort.

MATERIALS AND METHODS: The University of Pittsburgh Medical Center (UPMC) CoPath library was searched for "ameloblastoma," "keratocystic odontogenic tumor," and "odontogenic keratocyst" within the diagnostic line and diagnosis comment from 1/1/1990 to 7/1/2021. Exclusion criteria included maxillary lesions, unicystic ameloblas- toma, recurrent lesions, previously surgerized lesions, a diagnosis of Gorlin syndrome, and cases without three- dimensional imaging accessible through the UPMC system. The greatest dimension in the mesio-distal, bucco- lingual, and superior-inferior directions were obtained using Philips iSite/Imagecast. Location, locularity, cortical expansion, root resorption, tooth displacement, and association with impacted tooth(s) were assessed. The data was then de-identified and one-way ANOVA with multiple comparisons was conducted.

RESULTS: We identified 31 ameloblastomas and 45 KCOTs, with a mean age of 47.6 and 43.6 years and male to female ratios of 2.0:1 and 1.8:1, respectively. The average greatest cortical expansion (mm) of ameloblastomas and KCOTs were 12.3 and 5.3, respectively (p=0.0005).

CONCLUSION: We found that both ameloblastomas and KCOTs had a significantly greater mesio-distal dimension than buccolingual within the mandible. KCOTs had a significantly higher MD:BL and SI:BL ratios than ameloblas- tomas. The findings support our hypothesis and corroborate the limited published data of greater bucco-lingual growth in ameloblastomas.

Average lengths (mm)			
	Bucco lingual (BL)	Mesio distal (MD)	Superior inferior (SI)
Ameloblastoma	28.4	35.5	34.7
KCOT	15.4	32.8	30.3

Ratios of lengths			
	MD:BL	SI:BL	MD:SI
Ameloblastoma	1.35:1	1.29:1	1.07:1
KCOT	2.27:1	2.14:1	1.17:1

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A 10-Year Review of Intraoral Salivary Gland Tumor Diagnoses: Diagnostic Challenges and Inter-Observer Agreement

Monday, 11th April - 08:24: - Oral (Student/Resident)

Dr. Jessie Fuoco (Resident, Oral & Maxillofacial Pathology & Oral Medicine, University of Toronto, Toronto, ON), Ms. Mei Dong (Department of Biostatistics, University Health Network, Princess Margaret Hospital, Toronto, ON), Dr. Christina MacMillan (Department of Laboratory Medicine & Pathobiology, Mount Sinai Hospital, Toronto, ON), Dr. Ipshita Kak (Department of Laboratory Medicine & Pathobiology, St. Joseph's Healthcare Hamilton, Hamilton, ON), Dr. Bayardo Perez-Ordonez (Department of Laboratory Medicine & Pathobiology, University Health Network, Toronto, ON), Dr. Grace Bradley (Oral Pathology and Oral Medicine, Faculty of Dentistry, University of Toronto, Toronto, ON; Department of Dental and Maxillofacial Sciences, Sunnybrook Health Sciences Centre, Toronto, ON), Dr. Wei Xu (Department of Biostatistics, University Health Network, Princess Margaret Hospital, Toronto, ON), Dr. Marco Magalhaes (Oral Pathology and Oral Medicine, Faculty of Dentistry, University of Toronto, Toronto, ON; Department of Dental and Maxillofacial Sciences, Sunnybrook Health Sciences Centre, Toronto, ON)

Introduction: Salivary gland tumors (SGT) are a diverse group of neoplasms arising from major and minor glands. The oral cavity is the most common site for minor SGT (IMSGT) and these lesions frequently pose a challenge to the pathologist due to overlapping histopathological features and limited material for analysis. Our objective was to determine specific clinical and histopathological features associated with challenges in IMSGT diagnoses and pathologists' agreement.

Materials and Methods: We conducted a retrospective analysis of 248 IMSGT received between 2010-2019. We evaluated the diagnostic challenge of the cases by stratifying according to whether a definitive, favored, or indeter- minate diagnosis (challenging) was provided. Inter-observer agreement and concordance of biopsy diagnoses with the final diagnoses after tumor resection were evaluated.

Results: Of the 248 biopsies, 191 had a definitive diagnosis, 38 favored diagnoses, and 19 were indeterminate. The predominant diagnoses considered for the indeterminate category were pleomorphic adenoma/myoepithelioma (PA), polymorphous adenocarcinoma (PAC), adenoid cystic carcinoma (AdCC) and low-grade adenocarcinoma. Us- ing multivariate analysis of clinical features, younger patient age, smaller tumor size and larger biopsy size in- creased the likelihood of a definitive diagnosis (p=0.014, p=0.037, p=0.012). The inter-observer agreement for 68 representative cases was moderate overall (Fleiss's Kappa 0.575) and good for the 40 cases with a definitive diagno- sis (Fleiss's Kappa 0.66). Sixty-five biopsy diagnoses were matched with corresponding tumor resection diagnoses and found to show a good concordance (Cramer's V test 0.76). The discordant diagnoses predominantly involved PA, carcinoma exPA, PAC, AdCC and adenocarcinoma NOS.

Conclusion: Diagnostic challenges in IMSGT incisional biopsies were infrequent, especially if multiple pathologists were consulted. PA, PAC, AdCC and adenocarcinoma NOS were the histologic types more commonly posing diagnos- tic challenges. Younger patient age, smaller tumor size and larger biopsy are associated with a definitive diagnosis. This data highlights the importance of appropriate sampling in IMSGT.

Soluble immune biomarkers in head and neck squamous cell carcinoma can read the underlying histological tumor inflammatory profile and a novel regulatory role of Osteopontin

Monday, 11th April - 08:36: - Oral (Student/Resident)

Dr. Ioana Ghita (University of Maryland Baltimore), Dr. Vasileios Ionas Theofilou (University of Maryland Baltimore), Dr. John Papadimitriou (University of Maryland Baltimore), Dr. Robert Ord (University of Maryland Baltimore), Dr. Donita Dyalram (University of Maryland Baltimore), Dr. Joshua Lubek (University of Maryland Baltimore), Dr. Rania Younis (University of Maryland Baltimore)

Head and neck squamous cell carcinoma (HNSCC) is an immune-suppressive tumor with only ~15% response rate to immunotherapy. Further understanding of HNSCC inflammation is still needed.

We have previously described two HNSCC histological inflammatory subtypes (HIS) based on the extent of immune cells infiltrate in the tumor islands: inflamed (INF-HIS) and immune excluded (IE-HIS). The IE-HIS was characterized by higher soluble levels of the immune biomarker Sema4D (HsS4D) in plasma and higher Osteopontin (OPN) gene expression in the tumor. Here we investigated the association between the tumor HIS and the traditional plasma cytokines (CK) in blood of HNSCC patients.

Paired tumor tissue and peripheral blood samples were collected from ten HNSCC patients divided into five INF-HIS and five IE-HIS. We used five healthy donors (HD) and five autoimmune/chronic inflammatory conditions (AI/CI) patient samples as controls. ELISA-Luminex™ System was used for analyses of 40 traditional plasma CK. We used GraphPad Prism for statistical analyses.

For in vitro analysis rh-OPN protein was used to treat HNSCC cancer cell lines HN4 and HN6 (with IE-HIS phenotype). NOKSI (normal oral-keratinocytes), Leuk-1 and DOK (premalignant cell lines) were used as controls.

IL10, IL17A, IL-1RA and IP10 were higher in HNSCC patients compared to HD. Fractalkine, FGF2, GRO, CD40L were higher in the IE-HIS compared to the INF-HIS, while IL10 was higher in the INF-HIS. IL10 and IL6 were higher in the INF-HIS group compared to HD, while IP10/CXCL10 was higher in the IE-HIS.

Immunoblot indicated OPN significantly overexpressed in HN6 and HN4 compared to NOKSI, Leuk-1 and DOK. Treatment of HN6 with rh-OPN revealed dose dependent upregulation of Sema4D. This suggests regulation of the IE-HIS via OPN-Sema4D axis.

This research presents a novel approach showing that HNSCC can be subclassified according to soluble immune cytokine levels, with applications in personalized treatment and response to immunotherapy.

The diagnostic utility of PRAME in evaluating melanocytic lesions of the oral mucosa

Monday, 11th April - 08:48: - Oral (Student/Resident)

Dr. Jenna Marcinczyk _(New York Presbyterian Queens), Dr. Joonsung Yeom (New York Presbyterian Queens), Dr. Paul Freedman (New York Presbyterian Queens), Dr. Renee Reich (New York Presbyterian Queens)_

Introduction: PReferentially expressed Antigen in MElanoma (PRAME) is a cancer testis antigen that is overex- pressed in gonadal tissue and several cancers, including malignant melanoma. The use of PRAME as an IHC stain has become increasingly common in the evaluation of melanocytic tumors. Unlike IHC markers such as SOX10, S100, Melan-A, and HMB-45 that stain all melanocytes, the utility of PRAME is unique in its ability to differentiate between benign and malignant melanocytes. This finding has been documented in cutaneous melanocytic lesions; however, limited data exists on the application of PRAME IHC in oral mucosal melanocytic lesions. Pigmented le- sions in the oral cavity encompass a variety of entities that range from benign to malignant. Of particular interest is oral melanoma (OM) due to its very poor prognosis. It is important that any pigmented lesion without a clear etiology, especially those in high-risk sites like the maxillary gingiva and palate, be biopsied to rule out the possi- bility of melanoma. The aim of this study was to evaluate the usefulness of PRAME IHC as an adjunct marker in the evaluation of malignant potential in oral melanocytic lesions.

Materials and Methods: PRAME IHC staining was completed and evaluated on nine previously diagnosed melanocytic lesions that included melanoacanthoma, melanotic macule, melanocytic dysplasia, melanoma in situ, and melanoma.

Results: Positive nuclear PRAME IHC staining was noted in all cases of melanoma and melanoma in situ. Cases of melanoacanthoma, melanotic macules, and intraepithelial melanocytic dysplasia did not show nuclear overexpres- sion of PRAME.

Conclusion: Our findings are consistent with the current literature regarding PRAME IHC as it demonstrates pref- erential expression in malignant neoplastic melanocytic lesions. While more studies need to be done, PRAME IHC may be a useful ancillary tool in the evaluation of potentially malignant and malignant melanocytic lesions in the oral cavity.

ADENOCARCINOMA, NOS OF MINOR SALIVARY GLANDS OF THE ORAL CAVITY: A DWINDLING GROUP

Monday, 11th April - 09:00: - Oral (Student/Resident)

Dr. Lauren Ruddocks (University of Florida), Dr. Sarah Fitzpatrick (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Nadim M Islam (University of Florida)

INTRODUCTION: Salivary adenocarcinoma, not otherwise specified (ACNOS), is a diagnosis of exclusion. The incidence has been declining in recent years with the recognition of new adenocarcinoma subtypes. Recent analyses of ACNOS of the major salivary glands reclassified the majority of cases as salivary duct carcinoma after further test- ing; however, ACNOS of the minor glands of the oral cavity has been infrequently studied. The aim of this study is to collate and reclassify archival cases of ACNOS, based on current diagnostic criteria. **MATERIALS AND METHODS**: With IRB approval, all archival cases of ACNOS from the UF Oral Pathology Biopsy service between 1994 and 2021 were retrieved. Patient age, sex, biopsy location, and immunohistochemistry (IHC) results were recorded. All slides were reviewed and additional stains ordered based on histomorphology. Cases diagnosable as a specific adenocar- cinoma subtype were reclassified. **RESULTS:** A total of 22 cases were identified, all from the minor salivary glands. Mean patient age was 69 years, with a male predilection (n = 15, 68.2%). The palate was the most common location, followed by the buccal mucosa. P63 and p40 stains were useful in redefining 9 of the cases (41%) as polymorphous adenocarcinoma (PAC), followed by adenoid cystic carcinoma (n = 3, 13.6%), with other less frequent diagnoses comprising the remaining cases. 3 cases remained classified as ACNOS despite analysis. **CONCLUSION:** Most cases originally diagnosed as ACNOS from minor salivary glands of the oral cavity in this study were compatible with PAC upon further IHC; this justifiably contrasts with the studies of major salivary gland ACNOS. P63 and p40 IHC alone were helpful in reclassification of over half (54.5%) of all tumors and represent a useful diagnostic panel for the diagnosis of ACNOS of salivary glands of the oral cavity.

Reappraising the parameters that affect prognosis of oral leukoplakia with emphasis on inflammatory infiltration

Monday, 11th April - 09:12: - Oral (Student/Resident)

Dr. Vasileios Ionas Theofilou (Department of Oncology and Diagnostic Sciences, , University of Maryland, Baltimore), Dr. Maria Georgaki (Department of Oral Medicine & Pathology and Hospital Dentistry, School of Dentistry,National and Kapodistrian University of Athens), Dr. Efstathios Pettas ("Department of Oral Medicine & Pathology and Hospital Dentistry, School of Dentistry, National and Kapodistrian University of Athens), Dr. Rania Younis (Department of Oncology and Diagnostic Sciences, School of Dentistry, University of Maryland, Baltimore, USA), Prof. Nikolaos G. Nikitakis (Department of Oral Medicine & Pathology and Hospital Dentistry, School of Dentistry, NKUA, Athens, Greece)

Introduction: Oral leukoplakia (OL) is a prevalent oral potentially malignant disorder with histopathologic manifestations ranging from epithelial hyperplasia (not reactive) to varying degrees of oral epithelial dysplasia (OED). The presence and degree of OED affects the risk of malignant transformation (MT). On the contrary, microscopic characterization of OL stroma and its prognostic implications have not been extensively investigated, despite prior research indicating that inflammation initiates favorable prognosis of oral squamous cell carcinoma (OSCC).

Materials-Methods: Non-recurrent (N=20), recurrent (N=19) and transformed (N=13) OLs were retrieved and patient demographics and clinicopathologic characteristics were collected. The subepithelial connective tis- sue was characterized including the inflammatory pattern and extent, stromal density, and prominent fibrob- lastic/myofibroblastic reaction. Semaphorin 4D immunohistochemistry (previously shown to highlight OSCC- associated inflammation) was performed. Statistical correlations of clinical and histomorphologic findings with OL prognosis were performed using GraphPad Prism.

Results: Severe OED correlated with MT (p=0.0017), while age >55 years (p=0.0451) and size >2cm (p<0.0001) with OL recurrence or MT. Additionally, inflammatory infiltration (with or without exocytosis) was more commonly observed in transformed OLs (p=0.0109), while the extent of inflammation (p=0.0016) and Semaphorin 4D positive inflammatory cells (p=0.0095) correlated with recurrence or MT. Extensive inflammation was predominantly seen in non-smokers (p=0.0454) and moderate/severe OED grade (p=0.0267).

Conclusions: Our data suggest that inflamed OLs are more commonly encountered in specific patient subgroups (non-smokers), and correlate with OED grade and increased frequency of recurrence and/or MT. Clinicopathologic correlation is recommended to avoid OL misdiagnosis as other entities displaying subepithelial inflammatory in- filtration and equivocal MT risk (e.g. oral lichen planus). The complex roles and diverse prognostic significance of inflammation during the various steps of oral carcinogenesis (i.e. unfavorable in OL vs. favorable in OSCC) are also highlighted. It is suggested that incorporation of inflammation into future OED grading systems might be beneficial for optimal patient stratification.

Prognostic Role of Combined EGFR and Tumor-Infiltrating Lymphocytes in Oral Squamous cell Carcinoma

Monday, 11th April - 09:24: - Oral (Student/Resident)

Dr. Wattawan Wongpattaraworakul (Department of Oral Pathology, Radiology, and Medicine, College of Dentistry, University of Iowa, Iowa City, IA), Dr. Katherine Gibson-corley (Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN), Dr. Allen Choi (Department of Pathology, College of Medicine, University of Iowa, Iowa City, IA), Dr. Marisa Buchakjian (Department of Otolaryngology, College of Medicine, University of Iowa, Iowa City, IA), Dr. Emily Lanzel (Department of Oral Pathology, Radiology, and Medicine, College of Dentistry, University of Iowa, Iowa City, IA), Dr. Anand Rajand (Department of Pathology, College of Medicine, University of Iowa, Iowa City, IA), Dr. Andrean Simons (Department of Pathology, College of Medicine, University of Iowa, Iowa City, IA)

Background: Epidermal growth factor receptor (EGFR) is well known as a general prognostic biomarker for head and neck tumors, however the specific prognostic value of EGFR in oral squamous cell carcinoma (OSCC) is contro- versial. Recently, the presence of tumor-infiltrating T cells has been associated with significant survival advantages in a variety of disease sites. The present study will determine if expression of T cell specific markers (CD3, CD4 and CD8) would enhance the prognostic value of EGFR in OSCCs.

Methods: Tissue microarrays containing 146 OSCC cases were analyzed for EGFR, CD3, CD4 and CD8 expression using immunohistochemical staining. EGFR and T cell expression scores were correlated with clinicopathological parameters and survival outcomes.

Results: Results showed that EGFR expression had no impact on overall survival (OS), but EGFR-positive (EGFR+) OSCC patients demonstrated significantly worse progression free survival (PFS) compared to EGFR-negative (EGFR-) patients. High CD3, CD4 and CD8 patients had significantly better OS compared to low CD3, CD4 and CD8 patients respectively, but no impact on PFS. Combined EGFR+/high CD3 patients demonstrated significantly more favorable OS compared to EGFR+/low CD3 patients. CD3 expression had no impact on OS in EGFR- patients. Combinations of EGFR/CD8 and EGFR/CD4 expression showed no significant differences in OS among the expression groups.

Conclusion: Altogether these results suggest that the expression of CD3+ tumor-infiltrating T cells can enhance the prognostic value of EGFR expression and warrants further investigation as prognostic biomarkers for OSCC.

Mucoepidermoid Carcinoma Arising from the Surface Mucosa of the Oral Cavity: a Case Series

Monday, 11th April - 09:36: - Oral (Student/Resident)

Dr. Joonsung Yeom (New York Presbyterian Queens), Dr. Leigh Griffin (New York Presbyterian Queens), Dr. Paul Freedman (New York Presbyterian Queens), Dr. Renee Reich (New York Presbyterian Queens)

Introduction: Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, with an estimated frequency of 13% to 23% in minor gland tumors. We occasionally encounter MEC arising from the surface mucosa (SMEC) in intraoral biopsies, but these have not yet been described in the literature. This study aims to characterize the clinicopathological features of these tumors.

Materials and Methods: We reviewed the slides of 138 cases of intraoral MEC from the Oral Pathology Lab at NYPQ from 2010-2021 to identify cases in which the histology shows the tumor to be arising from the surface mucosa. The criteria for determining surface origin included 1) obvious transition of the surface epithelium or excretory duct epithelium into the underlying tumor or 2) full thickness involvement of the surface epithelium with mucous cells and cystic spaces with gradual transition to an infiltrating MEC. Cases in which the tumor appeared to grow into the surface mucosa or had equivocal histologic findings were not included.

Results: 14 of the 138 cases of intraoral MEC were found to be from the surface mucosa. For all MECs including SMECs, the palate was the most common site, followed by buccal mucosa, retromolar pad, and lower lip. Our MEC cases showed a strong female predilection (10:4), but SMECs showed an even greater female predilection (6:1). Interestingly, SMECs showed a bimodal age distribution with peaks in the fourth and seventh decades. Clinical appearance ranged from firm submucosal nodules to erythematous to ulcerated lesions. The histology showed surface mucosa transitioning to an infiltrating tumor containing classical features of MEC with mucous, epidermoid, and intermediate cells.

Conclusion: MEC can occasionally arise from the surface mucosa. These tumors have a striking female predilection, bimodal age distribution with peaks in the fourth and seventh decade, and variable clinical appearance.

The cytosine deaminating enzyme APOBEC3B and the chemical mutagen 4NQO promote oral carcinogenesis synergistically in vivo

Monday, 11th April - 09:48: - Oral (Regular)

Dr. Prokopios Argyris (Howard Hughes Medical Institute, University of Minnesota, Minneapolis, MN), Mr. Cameron C. Durfee (Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN), Prof. Rachel I. Vogel (Department of Obstetrics, Gynecology and Women's Health, University of Minnesota, Minneapolis, MN, USA), Prof. Reuben S. Harris (Howard Hughes Medical Institute, University of Minnesota, Minneapolis, MN)

Introduction: The APOBEC3 mutation signature is a substantial endogenous source of mutations in numerous human neoplasms. One of the enzymes responsible, APOBEC3B (A3B), converts DNA cytosines to uracils (C-to-U), which become immortalized as signature C-to-T/-G base substitution mutations. Oral and oropharyngeal cancers show elevated A3B mRNA and protein expression levels as well as higher proportions of APOBEC3 signature muta- tions. Here we asked how the endogenous mutagen A3B may combine with the exogenous mutagen 4NQO in oral carcinogenesis *in vivo*.

Materials and Methods: Six-week-old C57BL/6J mice engineered to constitutively express human *A3B* (n=12) were treated with 50 μg/ml 4NQO *ad libitum* in the drinking water for 16 weeks. The animals were reverted to normal water for an additional 6 weeks and sacrificed during the 22nd week. Wildtype C57BL/6J mice (n=8) served as the control group. The oral soft tissues and esophagi were harvested and examined histopathologically. A3B levels were confirmed by immunohistochemistry using a rabbit α-A3B mAb (5210-87-13). Statistically significant relationships were assessed using Poisson regression ($p<0.05$).

Results: Nontreated wildtype or A3B-expressing animals showed no oral lesions. 4NQO treatment caused intrao- ral and esophageal lesions, and A3B clearly exacerbated the tumorigenesis process (2.2 vs 3.8 lesions per animal, respectively; $p=0.047$). Microscopic features ranged from benign squamous papillomas to exophytic, papillary, high- grade, epithelial dysplasias and invasive squamous cell carcinomas (SCCs). Notably, A3B caused a 2-fold increase in the frequencies of oral and esophageal SCCs ($p=0.07$). By immunohistochemistry, all lesions in A3B-expressing animals showed strong and diffuse, nuclear positivity.

Conclusions: The mutagenic enzyme A3B functions synergistically with the chemical carcinogen 4NQO to promote oral neoplasia and increase the frequency of malignant tumors.

Break

Monday, 11th April - 10:00: - Oral (Regular)

Ms. Karen Benton *(12 Minute Break)*

Oral Session Break

Bone above the bone – First report of supraperiosteal ossificans.

Monday, 11th April - 10:12: - Oral (Regular)

Dr. Steven B. Whitaker (West Virginia University), Dr. Jerry Bouquot (West Virginia University), Dr. Safia Durab (Universiy of Texas, Houston), Dr. Lakshmi Garladinne (West Virginia University), Dr. Youseph Kassar (West Virginia University)

Background. Heterotopic bone is uncommon in the head & neck region, usually discovered a few months after trauma to muscle, tendons, or subcutaneous stroma. **Objective.** To present a previously unreported heterotopic bone lesion located just above alveolar periosteum. **Methods & Materials.** This is an observational study of a small convenience sample of cases. **Results.** We have noted dozens of examples of faint (usually), irregular, small to moderate-sized, globular radiopacities 1-3 mm above the periosteum, not connected to underlying bone. Almost always in long-edentulous alveolar crest sites, they typically showed a random pattern, but several presented as a thin radiopaque layer parallel to the cortex, similar to the "onion skinning" seen in Garré's osteomyelitis. All cases were asymptomatic and nonpalpable, with no abnormities of overlying mucosa. Many were noted years, even decades, after extraction, and were visualized with routine periapical films, but were better seen with cone beam computerized tomography (CBCT) imaging. Microscopic evaluation of incidentally biopsied examples showed rather immature bone with minimal osteoblastic activity (stagnant immature bone?), no visible osteoclasts, no miss- ing osteocytes, and a moderately dense, uninflamed but congested fibrous stroma. There was no dystrophic calci- fication, only bone, and the bone was visibly located some distance above the periosteum, without encapsulation. **Conclusion.** We report the first examples of heterotopic ossification within submucosal tissues of edentulous alve- olar sites. The lesional bone is viable, immature, inactive and nonencapsulated, without signs of inflammation. We suggest the descriptive diagnostic name "supraperiosteal ossificans" (SPO) for this entity.

Prevalence of oral leukoplakia in a single institutional biopsy service with histopathologic correlation

Monday, 11th April - 10:24: - Oral (Regular)

Dr. Mark Hochberg (Ohio State University), Dr. JOHN KALMAR (Ohio State University), Dr. Kristin McNamara (Ohio State University)

Introduction

Schwimmer introduced the term leukoplakia in 1877 to describe an oral white lesion. Controversy regarding its definition and role as a potential precursor to malignancy has existed for years. Most recently, leukoplakia has been described as "a white plaque of questionable risk having excluded known diseases or disorders that carry no increased risk for cancer." Its role as a "potentially malignant lesion" continues to be refined. The aim of this study was to evaluate the modern prevalence of oral leukoplakia and assess its correlation with histopathologic findings. *Materials and Methods*

An IRB-approved, retrospective review of five years (2014-2018) of accessions from the Ohio State University oral biopsy service (n=29,362) was performed to create a database. Lesions submitted as "leukoplakia", "keratosis" and "white" or "red-white" lesions (n=6,918) were initially selected. Histopathologic diagnoses were reviewed to elimi- nate "other" epithelial lesions as well as lesions of non-epithelial origin (n=3,739). The remaining 3,179 cases were analyzed for features of dysplasia or carcinoma with a separate category for red-white lesions, to evaluate historical differences in their biologic significance.

Results

The overall prevalence of leukoplakia was 10.8%. The prevalence increased with age to the eighth decade and was higher in males. Leukoplakia most frequently affected the lateral tongue. Most (61.2%) lesions were benign without evidence of premalignancy, while 30.5% of lesions showed low-grade dysplasia, 6.3% demonstrated high- grade dysplasia and 2.1% contained squamous malignancy at the time of biopsy. Only 35.1% of red-white lesions were associated with benign diagnoses, whereas 27.9% represented invasive squamous carcinoma.

Conclusions

Our results for the age, gender, and location of leukoplakia were consistent with previous studies; however, the overall prevalence and percentage of low-grade dysplasia were higher than previously reported. These findings also confirm the association of increased biologic risk with heterogeneous (red-white) lesions compared to their more homogenous counterparts.

Extra-gingival Pyogenic Granuloma: A 30-Year Retrospective Study

Monday, 11th April - 10:36: - Oral (Regular)

Dr. Paul Lewis (Private practice), Dr. Nagamani Narayana (UNMC College of Dentistry)

Objectives

1. To review the incidence/demographic data for extra-gingival pyogenic granuloma versus gingival pyogenic granuloma from 1987-2017 in the UNMC Biopsy Service archives.
2. To identify the diagnostic accuracy of clinicians at extra-gingival and gingival sites.

Methods

A retrospective study of oral, pathologically verified, pyogenic granuloma in the UNMC Biopsy Service during a 30-year period from 1987 to 2017 was conducted following an expedited UNMC IRB (389-18-EX). The reports were reviewed with demographic and clinical information recorded in Excel. Descriptive statistics (counts, percentages, means and standard deviations) were utilized. The independent sample t-test was used to compare the mean age by location (gingival vs. extra-gingival). The Kappa statistic was used to evaluate the agreement between the clinical impression and pathologic diagnosis.

Results

1938 patients were suitable for analysis. 56.40% of lesions were gingival and 43.49% were extra-gingival. The most common extra-gingival locations were tongue (15.48%), lip (10.84%), and buccal mucosa (6.30%). The median age for gingival lesions was 45 and that of extra-gingival lesions 53. The most common decade for gingival lesions was 40-49 and extra-gingival lesions, 50-59. 65.24% of lesions occurred in females and 34.76% in males. 37.67% of the clinical impressions included pyogenic granuloma. The weighted kappa agreement between clinical and histopathologic impressions of gingival lesions was 0.9439. The weighted kappa agreement between clinical and histopathologic impressions of extra-gingival lesions was 0.8884.

Conclusion

43.49% of pyogenic granulomas occurred at extra-gingival locations, which is higher than previously reported in the literature. The most common extra-gingival sites (tongue, lip, buccal mucosa) are in agreement with published reports. Extra-gingival lesions occurred in a slightly older demographic than gingival lesions with females predom- inating at both gingival and extra-gingival sites. Clinicians were more accurate at diagnosing lesions on the gingiva than on extra-gingival sites.

Single Cell RNA sequencing Reveals Pathogenic Driver Cells in the Salivary Glands of Sjogren's Disease

Monday, 11th April - 10:48: - Oral (Regular)

Dr. Blake M Warner (National Institute of Dental and Craniofacial Research), Mr. Thomas J. F Pranzatelli (National Institute of Dental and Craniofacial Research), Dr. Paola Perez-Riveros (National Institute of Dental and Craniofacial Research), Dr. Daniel Martin (National Institute of Dental and Craniofacial Research), Dr. Shyh-Ing Jang (National Institute of Dental and Craniofacial Research), Ms. Kalie Dominick (National Institute of Dental and Craniofacial Research), Dr. Eiko Yamada (National Institute of Dental and Craniofacial Research), Dr. John Chiorini (National Institute of Dental and Craniofacial Research), Dr. Margaret Beach (National Institute of Dental and Craniofacial Research), Ms. Eileen Pelayo (National Institute of Dental and Craniofacial Research), Dr. Zohreh Khavandgar (NIDCR), Dr. Alan N Baer (National Institute of Dental and Craniofacial Research)

INTRODUCTION

Sjogren's Disease(SjD) is a systemic autoimmune disease characterized by dry mouth and dry eyes. SjD etiology in- volves complex gene-environment interactions leading to pathogenic infiltration of self-reactive lymphocytes and autoantibodies generation. The immune-epithelial crosstalk and mechanisms driving the exocrine dysfunction are not defined. High throughput transcriptional analyses have broadened our understanding of the mechanisms of immune dysfunction in SjD. However, these 'bulk' approaches cannot uncouple diseases-specific changes in cellu- larity or cell-state simultaneously. We hypothesize that single cell transcriptomics can pinpoint effector immune cells and the pathogenic cell-cell interactions leading to parenchymal destruction and salivary hypofunction.
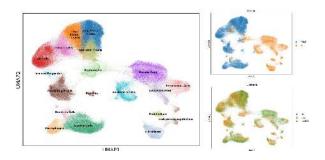
METHODS

SjD and control salivary glands(SG) and peripheral blood mononuclear cells(PBMCs) were used for single cell(sc) RNAseq, flow cytometry, and immunohistochemistry. To understand altered pathways in specific cell populations, we analyzed changes in expression and gene utilization across 7000 biological processes and annotated pathways from KEGG, GO, Reactome, and mSigDB.

RESULTS

An integrated and annotated scRNAseq atlas (Fig1 ~450,000 cells) from SG and PBMCs was used to determine changes in cell populations and transcriptional states. SjD SG had increased inflammatory cells (CD8+T-cells, B cells, IgG plasma cells), and loss of epithelial cells (acinar cells). Disease-specific differentially expressed genes, for example, components of MHC class I molecules, *B2M* and *HLA-B*, and interferon (IFN) stimulated genes, *IFI27*, were overex- pressed in SjD acinar cells; secretory markers (*MUC7*, *AQP5*) were decreased. In T cell clusters, functional annotation analysis revealed enrichment of pathways 'cell-to-cell adhesion', 'positive regulation of the Type I IFN response', 'T cell receptor signaling', and 'response to IFNγ signaling'. These results were confirmed using flow cytometry of MSG showing infiltrating CD8+T-cells express IFNγ and surface CD107a/LAMP1, indicating cytotoxicity.

CONCLUSIONS

scRNAseq resolves disease-specific transcriptional changes to a single cell resolution, partially explains symptoms of SjD, and raises new questions about effector immune cells and altered pathways in SjD.

Umap sg pbmc.jpg

Immuno-oncologic analysis of stage I oral squamous cell carcinoma relative to time of recurrence

Monday, 11th April - 11:00: - Oral (Regular)

Dr. Rania Younis (University of Maryland Baltimore), Dr. Ioana Ghita (University of Maryland Baltimore), Dr. Maria Georgaki (Department of Oral Medicine), Dr. Nicholas Wilken (Department of Oral and Maxillofacial Surgery school of Dentistry UMB), Dr. Robert Ord (University of Maryland Baltimore)

Introduction: The epidemiology of oral squamous cell carcinoma (OSCC) has shifted during the previous several years to include more of the non-smoker female patients. Stage I tongue SCC is more common in women and al- though overall survival rate for pT1N0M0 is very high, early or late local recurrence, or a new second primary may occur. Further investigation of the immuno-oncologic phenotypes of stage I OSCC can contribute to our understand- ing of this disease.

Material and methods: Following IRB approval, we collected pT1N0M0 OSCC tumor tissue retrospectively, for 25 patients treated in the Oral and Maxillofacial Surgery Department, University of Maryland Baltimore. The speci- mens included nine primary tumors with no recurrence. Six pairs of primary tumors with early recurrence (<48 months), and six pairs of primary tumors with late recurrence (≥48months) at the same location. Using Nanostring human IO-360 gene set and nsolver software, the differential pattern of expression of 700 immuno-oncologic genes implicated in tumorigenesis were investigated in the primary tumors and paired recurrence, as well as between primary tumors of early versus late recurrence.

Results: More than 2-fold changes in gene expression were noted in 13-45% of the studied genes in the primary tumors versus its early recurrent pair, and 15%-60% between the primary tumors and its late recurrence or metas- tases. Primaries of early recurrence (PER) had significantly higher level of gene expression compared to primaries of late recurrence (PLR). The lymphoid compartment, costimulatory signals, and MAPK pathway were significantly upregulated in the PER compared to PLR. PLR had more of hypoxia, matrix remodeling and metabolic stress path- ways compared to PER.

Conclusion: These findings suggest basal immuno-oncologic profiles of primary tumors that can be a spectrum for patient stratification to enhance personalized predictive measures and treatment of OSCC patients.

Flipped Pre-Doctoral General & Systems Pathology Curriculum

Monday, 11th April - 11:12: - Oral (Student/Resident)

Mr. Marc Generowicz (Touro College of Dental Medicine), Dr. Ronnie Myers (Touro College of Dental Medicine), Dr. Stephen Roth (Touro College of Dental Medicine), Dr. Aaron Yancoskie (Touro College of Dental Medicine)

Introduction: Development of critical thinking, clinical judgement and "the application of biomedical science knowledge in the delivery of patient care" (CODA standard 2-15) are critical in the education of undergraduate dental students. With the new case-based format of the INBDE, these skills come into even greater focus.

The flipped classroom format comprises provision of content for knowledge acquisition prior to in-class sessions, followed by use of in-class time to apply knowledge through active learning (e.g. group discussions and case-based learning). The educational literature has highlighted the multifaceted benefits of flipped classroom learning out- comes compared to conventional lecture format.

The Touro College of Dental Medicine D2 General and Systems Pathology course was designed in the flipped for- mat. In brief, students are required to preview video modules for knowledge acquisition, challenge a quiz at the beginning of in-class sessions to ensure completion of pre-work, apply knowledge acquired in the pre-work to case analysis in small groups sessions, and finally, respond to further quiz questions related to cases analyzed. Here we report our findings on the efficacy of this methodology over a 3-year span.

Materials & Methods: IRB approval was obtained to add 8 Likert scale questions to the course evaluation survey aimed at evaluating the effectiveness of this teaching methodology compared to the standard lecture format. Survey data from 2018-2020 was tabulated. NBDEI first-time pass rates were assessed.

Results: 191 students completed the survey over the 3 years. Overall, students preferred the flipped format and felt it promoted learning compared to traditional lecture (82.50% of students in 2018, 88.00% of students in 2019, and 72.82% of students in 2020). The first-time pass rates on the NBDEI were 2% above the national average.

Conclusions: Our study suggests the flipped format represents a promising model for providing pathology educa- tion to dental students.

Vesiculoerosive disease in the oral cavity: A five-year retrospective analysis of cases evaluated using direct immunofluorescence

Monday, 11th April - 11:24: - Oral (Student/Resident)

Ms. Krupa Thomas *(The University at Buffalo, State University of New York), Dr. Thomas Shanahan (Immco Diagnostics, Inc), Dr. Jill M Kramer (The University at Buffalo, State University of New York)*

Introduction: Vesiculoerosive diseases (VED) manifest in the oral cavity and the mucosal lesions that are charac- teristic of these conditions often result in reduced quality of life. Although VEDs are relatively rare diseases in the general population, the precise incidence and prevalence rates are not known due to the lack of large-scale population studies. Methods: We performed a retrospective database review of cases submitted to the Immco Di- agnostics biopsy service from 2015 to 2019 in which the diagnosis of VED was suggested or established using direct immunofluorescence (DIF). Objective: The objective of this study was to analyze demographic and clinical data for patients diagnosed with VEDs over this time period. Results: Consistent with the literature, we found these condi- tions were diagnosed most commonly in middle-aged females. Analysis of over 1,500 cases of VED cases diagnosed by DIF revealed that lichen planus was the most common condition, followed by mucous membrane pemphigoid. Pemphigus vulgaris and chronic ulcerative stomatitis were diagnosed much less frequently. Conclusion: Overall, our findings are in good agreement with the existing literature. Our study highlights the patient populations who are most at risk of developing an oral VED, and underscores the importance of DIF studies to facilitate diagnosis of these debilitating conditions.

Determining presence of ectopic germinal centers in minor salivary glands of patients with childhood Sjögren's disease.

Monday, 11th April - 11:36: - Oral (Student/Resident)

Dr. Shawki Abed (University of Florida), Dr. Seunghee Cha (University of Florida), Dr. Saja Alramadhan (University of Florida), Dr. Donald Cohen (University of Florida), Dr. Nadim M Islam (University of Florida), Dr. Sarah Fitzpatrick (University of Florida), Dr. Indraneel Bhattacharyya (University of Florida)

Introduction: A recent study of the University of Florida (UF) cohort with childhood Sjögren's disease (cSjD) has led to the detection of predominant CD4$^+$T and CD20$^+$ B cell infiltration with few Foxp3$^+$regulatory T cells in the infiltrates of cSjD. To further dissect the architecture and cellular compositions of the infiltrates, we investigated the presence of ectopic germinal centers (GC) in the minor salivary glands of cSjD.

Materials and Methods: cSjD cases (n=12) were selected for analysis from our institutional archives. The infiltrates of symptomatic non-cSjD patients (n=8), who failed to meet the 2016 SjD criteria, were also analyzed. Both cSjD and non-cSjD cases had at least 3 or more foci of over 50 lymphocytes. H&E and immunohistochemistry for GC-markers of CD10, CD21, and BCL6 were applied. The positive staining was graded in a blinded manner. The student t-test and Fisher's exact test were applied, where appropriate, with P-value <0.05 being significant.

Results: The number of CD10-positive cases was higher in cSjD than in non-cSjD without reaching a statistical sig- nificance (41% (5/12) vs. 37% (3/8). Most staining was weak and localized within the foci. No difference was found in the mean focus scores, age (range 6-17), or sex between CD10-positive and -negative cases. All specimens from both groups were negative for CD21. Bcl-6 was successfully performed on 13 specimens, with 69% (n=9) showing scattered positivity. Bcl-6 positivity was not significantly associated with cSjD diagnosis, focus score, or sex; how- ever, the mean age of Bcl-6 positive cases was 16.4 years as compared to negative cases at 12 years, with significance *p=0.017*.

Conclusions: This study identified minimal expression of CD10 and BCL-6, and absence of CD21, suggesting GC may be rare in cSjD. Possible aggregation of memory B cells, which lack surface CD21, in cSjD is under investigation.