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HEAD AND NECK CYTOPATHOLOGY

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The main areas sites amenable for cytopathology include lymph nodes, thyroid, major salivary glands especially parotid and soft tissue lumps and bumps. This presentation will focus on the major salivary glands. The aims are to discuss the utility of FNA in salivary gland pathology, review the cytologic diagnosis of the major pathological categories using case studies, illustrate a new entity (Mammary Analogue Secretory Carcinoma or MASC) and outline the use of molecular techniques in diagnosis.

UTILITY OF FNA IN SALIVARY GLAND PATHOLOGY

FNA of parotid gland is a generally accepted in the initial evaluation of salivary gland lesions. The goal is to categories lesions that are non-neoplastic (e.g. chronic sialadenitis, and which may not require surgical intervention), benign tumors (which are treated with limited resection), and, high grade tumors which require radical surgery (facial nerve sacrifice +/- neck dissection). The test is safe, fast, cost effective and accurate.

The accuracy rate of FNA from literature varies and reflects how negative, non-diagnostic or unsatisfactory specimens are scored. For example, Warthin tumor may be interpreted as chronic sialadenitis if the FNA lacks epithelial elements (false negative). The presence of mucus only on the FNA may not be representative of a low grade mucoepidermoid carcinoma since the aspirate is paucicellular (false negative). On the other hand, rare diagnostic cells may be overwhelmed by benign elements e.g. Hodgkin lymphoma. Given these scenarios, the sensitivity ranges from 64-100%, the specificity is consistently higher (94-100%) and non-diagnostic rates are in the range of 10-21%. In general, the main diagnostic difficulties are presented by cystic (hence paucicellular aspirates), lesions with overlapping features (e.g. cellular pleomorphic adenoma vs. basal cell adenoma vs. adenoid cystic carcinoma), lesions with low cytologic atypia (low grade MEC, acinic cell carcinoma) and uncommon lesions (lymphoma) [**Michael W Stanley Selected Problems in Fine Needle Aspiration of Head and Neck Masses Mod Pathol 2002; 15(3):342–350**]

In summary, FNA is helpful in diagnosis and planning surgery, should be used as part of clinical and radiological assessment, and, if necessary with intra-operative frozen section.

MAJOR DIAGNOSTIC GROUPS

As dictated by prevalence, these are pleomorphic adenoma, Warthin tumor and mucoepidermoid carcinoma (MEC). Acinic cell carcinoma will be illustrated because of its cytologic and histologic similarity to mammary analogue secretory carcinoma (MASC).

1. **Pleomorphic Adenoma**

Commonest tumor of the salivary gland. Diagnosis is straightforward when all three elements are present i.e. epithelial cells (mucinous, squamous, oncocytic), myoepithelial cells (spindle, plasmacytoid, clear) and stroma (myxoid, hyaline, chondroid). The stroma is characteristically fibrillar metachromatic material with embedded spindle myoepithelial cells. Diagnostic problems arise when there is a predominance of one of the three components. An excess of myoepithelial cells can be mistaken for myoepithelioma whilst a predominance of epithelial cells can be mistaken for adenoid cystic carcinoma or basal cell adenoma.

For a diagnosis of myoepithelioma, epithelial cells are by definition, absent. The presence of chondroid stroma is also disallowed.

Basal cell adenoma (previously monomorphic adenoma) is at the spectrum of epithelial rich tumor. Histologically, they are non-invasive and have various growth patterns. The membraneous (dermal analogue type) is one that is worth recognizing because of its association with Brooke-Spiegler syndrome (autosomal dominant, mutation of CYLD tumor suppressor gene, phenotype of multiple adnexal tumors such as trichoepitheliomas and cylindromas), its high incidence of recurrence (25%; or is it a new tumor due to multifocality) and increased malignant transformation rate. Cytologically, where metachromatic matrix is present, it is hyaline and not chondroid or fibromyxoid in texture. The differential diagnosis includes adenoid cystic carcinoma and basal cell adenocarcinoma. The cytologic diagnostic line should be broad (eg basaloid neoplasm). Adenoid cystic carcinoma has no squamous differentiation or basosquamous whirling and the basaloid cells generally surround the hyaline matrix. Take the clinical picture into consideration in that if there is facial nerve involvement, then a malignant basaloid tumor is a serious consideration.

2. **Warthin tumor**

For all practical purposes, this is exclusive to parotid/periparotid area and occurs in adults. It is the commonest bilateral tumor of the salivary gland. It is now accepted that this is not a neoplasm due to lack of clonality of the lesional cells **[Honda et al., Clonal analysis of the epithelial component of Warthin's tumor. Human Pathology 31; 2000]**. The etiology is proposed to be oncocytic metaplasia and proliferation which may be triggered by components in the cigarette smoke. The fact that is does not develop outside the parotid gives credence to the derivation being from parotid ductal remnants entrapped during lymph node development. No other salivary gland has intraglandular lymph nodes.

The diagnosis is straightforward if oncocytic cells, lymphocytes are seen in the background of cystic changes. Differential diagnoses in absence of these features include oncocytosis, oncocytoma, oncocytic carcinoma, acinic cell carcinoma, sialadenitis, intra parotid lymph node and lymphoma. Particular attention should be paid to the flat sheets of non-overlapping cells in Warthin. The cytoplasm is finely granular and not waxy or coarsely granular. Metaplastic mucous or squamous cell may be seen as well as degenerate pleomorphic/pyknotic cells with orangeophillic cytoplasm. These features may mimic squamous cell carcinoma, mucoepidermoid carcinoma or acinic cell carcinoma, and are a recognized pitfall of cytologic diagnosis.

3. Mucoepidermoid carcinoma (MEC)

MEC is the most common salivary gland neoplasm across all ages and gland locations. It covers the spectrum from low to high grade. Grading is particularly associated with prognosis in the case of MEC arising in the parotid gland, not so much in the submandibular or other minor salivary glands.

For a confident diagnosis of MEC, the FNA findings should show a mixture of cell types (epidermoid, mucous and intermediate cells). With increasing grade, the proportion of epidermoid cells increases. Conversely, lower grade tumors have more mucous cells than epidermoid cells and tend to be more cystic.

The mucous cells have abundant vacuolated cytoplasm which may be foamy, have micro vesicles of mucin or be like a goblet cell. Epidermoid cells have abundant distinct eosinophilic cytoplasm. The present of a mucin droplet within an epidermoid cell is highly characteristic of a MEC. On the other hand, keratinizing squamous cells are not typical and should lead to other diagnostic considerations (squamous metaplasia in chronic sialadenitis, squamous metaplasia in lymphoepithelial cyst, squamous metaplasia in Warthin tumor, metastatic (adeno)squamous carcinoma). Intermediate cells are the progenitor cells which can differentiate into epidermoid or mucous cells. They have high nuclear cytoplasmic ratio and are most easily identified by their scant cytoplasm.

4. Acinic cell carcinoma (AciCC)

Acinic cell carcinoma is the most common bilateral malignancy of the parotid gland. In children, it rank as the second most common malignancy after MEC. In adults, it is the third following MEC and adenoid cystic carcinoma.

Like adenoid cystic carcinoma, acinic cell carcinoma has a protracted course and can metastasis to lung and bone. Unlike adenoid cystic carcinoma, however, acinic cell carcinoma also metastasizes to lymph nodes.

Recently, it has transpired that MASC contributed towards some of the cases histologically diagnosed as AciCC. It is because of this that both are being reviewed here.

The diagnostic features are of a hyper cellular aspirate composed of cells with abundant cytoplasm which may range from foamy to coarsely granular and basophilic. The cell clusters are overlapping and crowded with, typically, single cells as well as bare nuclei in the background. At least some of the cells should have granular, zymogen rich cytoplasm since degranulation may impart a clear or foamy appearance. In distinction to MASC, the cells are S100 and mammoglobin negative. They also lack the diagnostic translocation t(12;15) ETV6-NTRK3.

5. Mammary Analogue Secretory Carcinoma

This entity was first described in 2010 by Skalova who noticed the morphological similarity to juvenile secretory carcinoma of the breast. It is most frequently found in the parotid but has also been reported in the submandibular and minor salivary glands. Unlike juvenile secretory carcinoma of the breast, MASC occurs in adults (mean age 44yrs, range 14-77) with a M:F ratio of 1:1. Similar to AciCC, MASC can metastasize to lymph nodes. Follow up data is being accrued so as to further define this entity clinically and therapeutically.

Cytologically, it can be regarded as a zymogen poor AciCC. Characteristically, the cells are dispersed similarly to those from AciCC. Mucin may be seen both intra and extra cellularly. The differential diagnosis are MEC, adenocarcinoma NOS, AciCC. Diagnosis requires demonstration of the t(12;15) ETV6-NTRK3. This translocation is also found in mesoblastic nephroma and congenital fibro sarcoma; it encodes a chimeric tyrosine kinase protein and presents an attractive potential therapeutic target. Immunocytochemical distinction from AciCC may be used as a surrogate i.e. S100 +, Mammoglobin +.

6. **Metastatic carcinoma.**

The most frequent are head and **neck squamous cell carcinoma** (SCC) and malignant melanoma (MM) which metastasize to the intra parotid lymph nodes. PRIMARY SCC IS A DIAGNOSIS OF EXCLUSION.

Beware of mimics of SCC which include degenerative orangeophillic cells in benign cystic lesions such as Warthin tumor and lymphoepithelial cysts, atypical metaplastic squamous cells in chronic sialadenitis and radiation sialadenitis. For a confident diagnosis, require lots of abnormal squamous cells in various stages of differentiation. Otherwise, report as atypical squamous cells and suggest excision of the lesion.

20% **of malignant melanoma** arise in the head and neck site. Of these, 80% are cutaneous with the remainder occurring in ocular and mucosal sites. The FNA, if adequate, is clearly malignant and if pigmented, is diagnostic.

<u>ANCILLARY MOLECULAR TESTING IN SALIVARY GLAND TUMORS</u>

Four salivary gland neoplasms have been shown to have distinct translocations. These promise diagnostic purity of the categories and perhaps potential therapeutic targets. These tests may also define the clinical course in terms of prognostic information.

MASC has already been discussed above. 70% of **MEC** contain a translocation t(11;19) MECT1:MAML2. This translocation has been associated with lower risk of local recurrence, metastases and tumor related death compared with cases where the translocation was not found. The fusion protein has been characterized although its precise function is not clear. 65% **of Adenoid Cystic carcinoma** studied by FISH were found to harbor a t(6;9) MYB:NFIB . This fusion protein is of unknown function and whether it is associated with prognosis is not know at this time. **Hyalinizing clear cell carcinoma (HCCC)** is a low grade salivary gland tumor with squamous and occasional mucinous differentiation. In over 80% of cases, a t(12:22) EWSR1:AFT1 has been found by RT-PRC or FISH. This has highlighted variant

morphologies such as mucous differentiation. It shares similar morphology with clear cell odontogenic tumor which bears the same translocation and differs only by location.

In conclusion, we are now entering the era of classification of salivary gland tumors that takes into account the clinical, morphologic as well as molecular data. The new WHO book will most likely reflect this and we should be ready to embrace it into our daily diagnostic practice in cytology as well as surgical pathology.