FNA of Salivary Gland: Problems and Pitfalls

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Introduction

Of all the anatomical sites at which to perform a fine needle aspiration biopsy (FNAB), perhaps the head and neck area is the most complex. This area is the home of many structures, all of which have the potential to produce benign and malignant tumors. The salivary glands are only one such system; others are lymph nodes and peripheral nerves. This area can also be the site of metastases from cancers arising above and below the clavicle including central nervous system and skin adnexal neoplasms.

FNAB is a useful technique for evaluating masses suspected of being of salivary gland origin. In most instances, the masses are not neoplastic; inflammatory and benign tissue masses account for over 50% of all such lesions. The overwhelming majority of neoplastic lesions are benign. Carcinomas and lymphomas are found in less than 10% of salivary gland FNABs. Biopsies that indicate an inflammatory or benign condition obviate the need for a surgical biopsy. When neoplastic tissue is revealed, its morphology may dictate the urgency and extent of any subsequent surgery. Some critics (Batasakis, J.G., Ann Otorhinolaryngol 101: 185, 1992) argue that while FNAB is particularly applicable to lesions of the head and neck, it is not so diagnostically efficacious for sampling major salivary glands. Dr. Batasakis believes that certain histopathologic effects secondary to FNAB create tissue artifacts that renders the sample difficult to interpret. However, another article (Cramer, H., Layfield, L., Lampe, H., Ann Otolrhinolaryngol 102: 483, 1993) effectively refutes Dr. Batasakis’ contention. In this review FNAB was shown to be superior to physical examination, radiological examination and core biopsy for diagnosing masses in salivary glands. The accuracy rate of salivary gland FNABs has been reported in only a few large case series. Several factors have hindered attempts to evaluate the procedure’s sensitivity and specificity. In many series, the salivary gland is included in other head and neck lesions, and there is little agreement among authors over what constitutes an unsatisfactory, atypical, or histologically unconfirmed case. These factors aside, the sensitivity of FNAB is approximately 90% and the specificity, approximately 95%. The ability to specifically designate what type of benign or malignant neoplasm is present, however, is somewhat more problematic. The ability to specifically designate a benign neoplasm is about 90% and for malignant neoplasms, about 75%. While these statistics may not seem particularly good, it is important to realize that they are no lower then those for frozen section.

FNAB specimens from salivary gland are difficult to diagnose for a number of reasons, including problems in sampling as well as difficulty in histological classification. A major roadblock in the histologic classification of salivary gland neoplasms is that the majority of them arise from the same cell lines (epithelial and myoepithelial). Added to this fact is the ability of these cells to undergo a variety of metaplastic changes (squamous, mucinous, sebaceous, oncocytic, and chondroidal). With all the difficulties in classifying salivary gland neoplasms histologically, it is easy to imagine how difficult it
is to be absolutely correct on a cytologic sample. I have found that instead of trying to memorize long lists of various entities, it is better to approach salivary gland lesions by generating a differential diagnosis outline. An approach that has worked for many observers is to place the lesions in five diagnostic categories based on the cell type and extra-cellular material. Within the categories, entities can be further defined by assessing the amount of nuclear abnormalities present. As with neoplasms in all body sites, it should be remembered that some may not arise from the organ itself but may be extrinsic to it and merely in be close proximity (Figures 1-5).

Before discussing lesions of the salivary glands, I will briefly review their normal histology and cytology. The three major salivary glands are the parotid, the submandibular, and the sublingual. The minor salivary glands occur as numerous microscopic foci throughout the oropharynx. An FNAB of the salivary gland should yield both glandular (acinic cells) and ductal elements; there may also be fat. The acinic cells are most often seen in cohesive ball-like structures, whereas ductal elements occur as cohesive orderly sheets or, more rarely, as tubules. Often, the acinic cells appear as a background field of bare nuclei. The acinic cells are either serous or mucinous in type. Elongated myoepithelial cells may be seen attached to the epithelial elements. The cytoplasm of the serous cells is finely granular or somewhat foamy compared to the more dense cytoplasm of the ductal elements.

Lesions
1. Myxoid-Hyalin Lesions
   The first differential diagnosis list consists of those lesions that produce myxoid or hyalin-like extracellular material (Table 1). These include benign mixed tumors (BMT), adenoid cystic carcinoma (ADCC), carcinoma ex (EXBMT), and polymorphous low grade adenocarcinoma (PLGA). Lesions that arise within the region, but are not of salivary gland origin include schwannoma, myxoma, myxoid lipoma, and myxoid neurofibroma.

   BMTs account for approximately 75% of all salivary gland tumors. They consist of an admixture of epithelium, myoepithelium, and stroma. The FNAB will contain fibrillar myxoid stroma amidst a uniform population of epithelial cells that occur singularly and in cohesive groups. The stroma will stain red to purple with the May-Grunwald/Giemsa stain. On a Pap stain this myxoid substance varies in color but has a fibrillar nature. Both the epithelial and the stromal components can show metaplastic change. In the epithelial component there may be squamous metaplasia, oncocytic change, or mucin production. The stromal component, while most often myxoid, may appear more chondroidal. The ratio of epithelium to stromal components varies widely. When the epithelial component predominates, the tumor may be referred to as a monomorphic adenoma (MA). Diagnostic difficulties arise when there are atypical changes in the nuclei, such as variation in size and shape. In this event, the possibility that the lesion is a carcinoma arising in a BMT should be considered. Another diagnostic problem occurs when histologic sections of the tumor contain areas that mimic ADCC. One way around this difficulty is to sample the lesion well and find areas that
contain obvious BMT-like changes. Many observers believe that if the lesion is well sampled, any area containing elements of classic BMT should overrule any smaller areas of worrisome epithelium or structures. It should also be remembered that ADCCs often invade the facial nerve and cause pain or paralysis. It has been advocated that one not make a diagnosis of ADCC unless there is evidence of nerve involvement.

ADCC accounts for about 1% to 2% of all salivary gland tumors. This tumor is found most often in the parotid gland; however, it accounts for the majority of malignant tumors involving the palate. This is an indolent tumor with a predilection for infiltrating nerves. ADCCs can be difficult to diagnose because of the relatively small size of the tumor nuclei, good cell-to-cell cohesion, and their rather bland appearance. There are three histologic patterns: cribriform, tubular, and solid. The cribriform pattern is the easiest to diagnose because of the presence of metachromatic stromal material surrounded by small uniform nuclei. Unfortunately, however, similar areas can be found in BMTs and PLGA. A careful comparison of ADCC and BMT will reveal subtle differences between them. The ADCCs tend to have clusters of cohesive cells that are smaller and have less cytoplasm than the cells of BMT. When the cells do occur singly they have only a small amount of cytoplasm, compared to BMTs, which have moderate amounts of cytoplasm and eccentrically-placed nuclei.

PLGA is a recently described neoplasm. This tumor is found mainly in women and almost always in the hard palate. They consist of cells that can be basaloid or cuboidal and may form glandular, solid, or cribriform structures within a myxoid stroma. Thus, this neoplasm may be impossible to diagnose cytologically as its features overlap considerably with those of other tumors.

The most common extrinsic neoplasm in this group of lesions is the schwannoma. Schwannoma is characterized by spindle cells embedded in an acellular matrix. This matrix lacks the fibrillar qualities seen in a BMT. In schwannoma, two patterns of growth are noted, Antoni A and Antoni B.

II. Basaloid Lesions
The differential diagnosis of basaloid lesions includes a number of intrinsic and extrinsic neoplasms that are quite difficult to separate (Table 2). Distinguishing these lesions from each other can be frustrating as neither the cytology nor the histology can always allow for their differentiation. The extrinsic lesions are from that all-too-familiar group of neoplasms called “small round blue-cell tumors” which, in order to identify correctly, require a full clinical history as well as immunohistochemical, cytogenetic studies. The primary salivary gland tumors are just as difficult to distinguish from each other, as most have overlapping cytologic and immunohistochemical properties. The intrinsic basaloid lesions include basal cell adenoma (BCA), basal cell carcinoma (BCC), the solid variant of ADCC, PLGA, and small cell undifferentiated carcinoma.
BCA is an uncommon primary tumor of major salivary glands. Histologically, the tumor consists of uniform basal cells that occur in a trabecular, tubular, solid, papillary, or membranous configuration. The tumor cells are separated by a non-myxoid stroma similar to that seen in many BMTs. Cytologically, smears are cellular and the neoplastic cells occur as solid groups, occasionally as branching chords. Single cells are not prominent, but when they do occur, they have only a scant amount of cytoplasm. The nuclei are smaller and darker than those seen in the monomorphic variety of BMT. Nucleoli are not present. There is a malignant variant of this neoplasm (BCC) that can only be identified on histologic sections by the demonstration of invasion or metastases. Cytologically, these tumors can be confused with the cellular variant of BMT. From the few cases described in the literature, and in our own experience, it seems that the distinguishing feature of this tumor is the fact that the tumor cells are quite cohesive compared to BMT. On cytologic samples these cohesive groups often show a smooth communal border and nuclear palisading at their periphery. The membranous type has a hyaline like band around the tumor cell clusters. The nuclei are smaller and more hyperchromatic than their counterparts in monomorphic BMTs.

The other neoplastic conditions that may have a similar appearance to BA are the solid variant of ADCC and PLGA. The overlap in the this group is so significant that when the cytologic pattern presents itself, we will only offer the differential diagnosis list and recommend histologic examination for specific subtyping.

III. Oncocytoid Lesions

Compared to the previous group, lesions that show oncocytic change are relatively easy to diagnose. In addition to oncocytic change, this group of lesions exhibits varying degrees of nuclear abnormality. This feature can, at times, help in subclassifying the lesions.

Warthin’s tumor (WT) is a common tumor of the salivary gland, which is surpassed only by BMT in prevalence. This tumor arises almost exclusively within the parotid and is bilateral in approximately 10% of cases. On clinical examination, this tumor has a doughy consistency and, when aspirated, a small amount of mucoid material is often harvested. Microscopic examination shows this lesion to be a cyst in which there is papillary growth. The papillations are covered by a double layer of oncocytic epithelium. Surrounding the lesion, and an integral part of many of the papillations, is lymphoid tissue with germinal centers. The origin of these tumors is felt to be the proliferation of entrapped salivary gland ducts within intra- and periparotid lymph nodes.
Aspirates from WTs consist of monolayered sheets of oncocytic cells with good cell borders. Mast cells often accompany the epithelial sheets. The lymphoid elements usually do not occur as single cells but in clumps, clusters, and tangles. The cyst content may be thick or thin and contain amorphous debris. The oncocytic epithelium often undergoes squamous and/or a mucinous metaplasia. As one can imagine, if this occurs, the diagnosis can be difficult as this neoplasm may be confused for a cystic squamous cell carcinoma or a mucoepidermoid carcinoma.

Oncocytomas are infrequently occurring benign neoplasms. They may be found in any of the major salivary glands; however, they are more common in the parotid. The cytologic pattern of these tumors will show groups and single cells with abundant, granular, eosinophilic cytoplasm. Nuclei tend to be round and nucleoli are present. The individual cells and sheets from an oncocytoma bear a striking resemblance to WTs; however, in the former, there are no lymphocytic cells. Oncocytic metaplasia is known to occur in BMTs; however, the presence of the fibrillar metachromatic stroma in BMT identifies the lesion. Once the characteristic oncocyte has been identified, it is probably not all that important to precisely subclassify the lesion, as these oncocytic lesions are simply surgically removed. It is more important to separate out of this group the acinic cell carcinoma, which may require a different surgical approach.

Acinic cell carcinomas occur in both major and minor salivary glands. They are rare, constituting only about 1% of all salivary gland tumors. They are not usually aggressive; however, metastases have been reported. FNAB will yield clusters and aggregates of cells bearing a striking resemblance to normal acinar cells, especially in the usual well-differentiated type. The well-differentiated tumors may resemble renal cell neoplasms, but will be PAS positive and diastase resistant. These cells are often difficult to distinguish from normal salivary glands; however, true acinar structures, ductal cells, and fat are missing. The poorly differentiated varieties have hyperchromatic, pleomorphic nuclei with large nucleoli. There is a tendency for the cells to be larger than their normal counterparts. In well-differentiated tumors, the nuclei are small, round, and uniform, and may have a central nucleolus. The cytoplasm is abundant and finely vacuolated to granular, which helps to distinguish them from the more uniformly granular oncocyes.

The spectrum of extraglandular oncocytic neoplasms covers a wide range of lesions, including benign tumors such as paraglanglioma, carcinoid, granular-cell tumor, and rhabdoid tumors. The accurate diagnosis of these neoplasms depends in part on clinical presentation, including age of onset, and immunohistochemical studies. Malignant oncocytic neoplasms are mainly metastatic tumors such as renal cell carcinoma, melanoma, medullary carcinoma, Hurthle cell carcinoma and hepatocellular carcinoma. Previous clinical history and stains may be required to confirm the diagnosis.
IV. Lymphoid Lesions

Up to this point we have discussed lesions that were of epithelial or stromal origin; we will now look at benign and malignant lymphoid lesions. From a practical standpoint, it is more important to understand the three most frequently-occurring non-neoplastic lymphoid masses that are found either in, or adjacent to, the salivary glands. These are chronic sialadenitis (CS), benign lymphoepithelial lesion (BLEL), and intra- or perisalivary gland lymph nodes.

The parotid and submandibular glands are surrounded by lymph nodes. The parotid gland also has intraglandular lymph nodes, over 90% of which are in the superficial lobe. It should not be surprising that any lymph node enlargement in or near the salivary glands may appear clinically as a salivary gland tumor.

CS may occur in any salivary gland; however, the submandibular gland is the most frequent site. CS most often arises secondary to ductal obstruction. The obstruction may be a result of increased viscosity of secretions, sialolithiasis, or ductal damage. The submandibular gland is most susceptible due to the tortuous path of its duct and subluxation of the gland with aging. Ductal obstruction causes an inflammatory reaction, which leads to atrophy of the glandular epithelium and replacement by fibrous tissue. The ductal epithelium may undergo metaplastic changes, including squamous, oncocytic, and mucinous type. Due to ductal dilatation, cysts may form.

The cytologic picture of CS depends on when in the course of the disease the aspirate is obtained. The smears consist of a mixed population of lymphocytes, with clusters of ductal epithelium and acinar groups. The background may contain mucin or proteinaceous material. The number of acini lessens over time and may eventually disappear entirely. If squamous or mucinous metaplasia is very prominent, it can easily be mistaken for mucoepidermoid carcinoma. The FNA biopsy of CS is sometimes painful, unlike those of neoplasms, which are usually relatively painless.

Histologically, benign lymphoepithelial lesions (BLEL) consist of a marked lymphocytic infiltrate of a salivary gland and/or a lacrimal gland. Lymphoid structures with germinal centers and epimyoepithelial islands, which consist of basal epithelial cells and modified myoepithelial cells, are also present. As the lesion progresses, the acini atrophy, leaving only the ducts, lymphoid tissue, and epimyoepithelial islands.

BLEL is most often a result of autoimmune sialadenitis. Two forms are recognized: Mikulicz’s disease and Sjögren’s syndrome. The former is a localized process and the latter is systemic. Other causes include HIV infection.

The FNAB of BLEL is usually moderately to abundantly cellular. The components include a heterogeneous population of lymphoid cells such as one
would see in a hyperplastic lymph node, epimyoepithelial islands, ductal epithelium, and atrophic acini, which may be absent in late stages of the disease.

A source for a misdiagnosis in this group are neoplastic lesions that may be associated with lymphocytes (WT, lymphoepithelial carcinoma or metastasis to an intra- or periparotid lymph node). If these lesions are not well sampled, the non-lymphoid component may be missed.

V. Squamoid Lesions
This last category will concern the differential diagnosis of those lesions that have minimal to moderate cytologic abnormalities (Table 5). It will not be necessary to discuss obvious carcinomas, as they have high grade nuclear changes.

Squamous cells can be obtained from within the salivary glands, as well as from extraglandular structures. The lesions can be either neoplastic or non-neoplastic and tend to be cystic.

Non-neoplastic lesions are usually either retention cysts or mucoceles. These lesions are found most often on the floor of the mouth or the lower lip. A biopsy of these will often produce a small amount of watery to mucoid fluid. The cellular content comprises mainly macrophages, but sparse epithelial elements are also present. If secondarily infected, acute and chronic inflammatory cells will be found.

Benign congenital cysts extrinsic to the salivary glands include branchial cleft, thyroglossal duct, thymic, and dermoid/epidermal inclusion cysts. Malignant cystic lesions include metastatic well-differentiated cavitary squamous cell carcinoma.

Branchial cleft cyst is the most common cystic lesion found in the neck. Patients tend to be under 30 years of age; these lesions are rarely seen in older patients. Their exact origin is uncertain, i.e., could these represent true congenital remnants from the branchial clefts or do they merely represent cystic change in salivary gland ductal inclusions in perisalivary gland lymph nodes. Branchial cleft cysts are usually found near the anterior part of the sternocleidomastoid muscle, most often in the upper third of the neck and close to the parotid. Aspiration yields a turbid material that tends to contain only a few epithelial elements, which range from immature metaplastic to intermediate and superficial type squamous cells. Other cells include macrophages and scatterings of lymphocytes. In addition to the squamous elements, occasional glandular cells with mucin or cilia may be seen. Keratinization is not usually prominent.

The differential considerations for these lesions include metastatic, well-differentiated squamous cell carcinoma, or metastatic mucoepidermoid carcinoma. The former is usually easily identified due to the finding of
pleomorphic forms and a careful search for less than well-differentiated cells. The latter may be most difficult to distinguish from a benign lesion.

An important entity to discuss at this point is the benign epithelial cyst as seen in the AIDS patient. The lesion is thought to occur either due to obstruction of ducts by lymphoid hyperplasia or destruction of ducts due to cell-mediated immunity. HIV-associated parotid cysts are often multiple and bilateral. The lining is most often squamous, but glandular epithelium has been described. The cellular elements are most often quite bland and include inflammatory cells as well as the above-mentioned epithelial cells. While not common, lymphomas have been noted to develop in this condition. It is also important to remember that these lymphoepithelial cysts may be the presenting clinical finding of an HIV-positive person.

Other lesions that may, on rare occasions, present as cystic structures in close proximity to the salivary glands include metastatic thyroid cystic papillary carcinoma, thyroglossal duct cysts, and thymic cysts.

Cystic lesions are well-known sources of false-negative diagnoses. Ways to avoid this unfortunate occurrence include rebiopsying any residual cyst mass after draining. It has also been the experience of most observers that if cysts recur, it is wise to remove them. False-positive diagnoses are also a problem with cystic lesions. One of the most common settings for this diagnostic pitfall is in a cyst with extensive squamous metaplasia. If it becomes irritated for any reason, such as by infection or radiation, considerable pleomorphism may occur and one would be tempted to entertain a diagnosis of squamous cell carcinoma.

Mucoepidermoid carcinomas are responsible for slightly less than 10 percent of salivary gland tumors. The parotid gland is the most common site. These tumors are amongst the most difficult to diagnose on FNA material because the cellular components (squamous epithelium, glandular epithelium and mucin) vary greatly in their amount and their degree of differentiation. The well-differentiated variety is most difficult to delineate as a neoplasm and the poorly-differentiated tumor is difficult to tell what type of neoplasm it is. Three cytologic features have been cited as being more predictive of mucoepidermoid carcinoma: intermediate cells, clusters of overlapping nuclei, and squamous cells. The squamous elements that are present rarely show obvious keratinization. The exact definition of intermediate cells is confusing in the literature. These cells have been described as being small basal-like cells all the way up through immature squamous metaplastic cells.

Poorly cellular samples with only mucin and a few glandular and squamous elements may often be incorrectly identified as a benign condition such as mucocele or CS. At other times there may be mucin present and a moderate amount of squamous and/or glandular epithelium. This again may only represent
CS. The wisest course in samples that are modestly cellular with minimal atypia and mucin production is to recommend surgical removal.

Salivary duct carcinoma (SDCa) is uncommon adenocarcinoma of salivary gland origin that has histological features seen in high-grade ductal breast carcinoma with comedo necrosis. These are aggressive tumors with facial nerve dysfunction, nodal/distal metastasis, and local recurrence. There is not a pathognomonic cytological presentation for this neoplasm. They yield on FNA a cellular sample with cell groups that may be flat or in a cribriform pattern, single polygonal shaped cells with dense/granular cytoplasm, large pleomorphic eccentrically placed nuclei, and comedo necrosis. In most cases it is best to offer a differential diagnosis that includes poorly differentiated carcinoma (squamous or adeno), high-grade mucoepidermoid carcinoma, moderately differentiated squamous carcinoma, and SDCa. Expression of androgen receptor by immunohistochemistry has been suggested as useful in identifying this latter neoplasm.

From this short overview, it can be seen that the FNAB diagnosis of salivary gland lesions can be fraught with problems and pitfalls. It is hoped that this complex area will make more sense when one thinks about these lesions in the context of the five described groups.

REFERRENCES


