Salivary Fine Needle Aspiration Biopsy

3

William R. Ryan, A. Sean Alemi, and Annemieke van Zante

Key Points

1. Salivary surgeons must be aware of the benefits and limitations of fine needle aspiration (FNA) in the diagnosis of salivary pathology.
2. Immediate assessment of FNA adequacy and quality by an experienced cytopathologist may reduce the time to diagnosis.
3. FNA with ultrasound guidance may improve specimen adequacy and diagnostic yield.

Impact of Salivary FNA

Information obtained from FNA can directly influence management of salivary masses. One study calculated a degree of impact of cytologic diagnosis as changing management at least 35% of the time [1]. FNA can help guide clinicians to avoid surgery for conditions, such as lymphoma or inflammatory lesions, and implement conservative observational approaches for certain benign tumors (particularly in the following situations: frail patients at a higher risk for complications with surgery under a general anesthetic, some asymptomatic patients, and patients hesitant to undergo surgery). FNA cytology can contribute a specific or differential diagnosis allowing appropriate preoperative counseling regarding the extent of resection, facial nerve management, the need for neck dissection, and the degree of urgency. Preoperative cytologic diagnosis can also mentally prepare a patient for the final diagnosis based on surgical pathology, particularly when malignant.

FNA Technique

A cytopathologist, surgeon, or radiologist may perform FNA depending on the clinical situation and institutional policy. Identification of the target is essential to successful aspiration. Nonpalpable, ill-defined, or deep lesions are best aspirated under ultrasound or CT (computerized tomography) guidance. Superficial nodules are best done by palpation or ultrasound guidance. Parapharyngeal or some deep lobe parotid lesions, when not easily visualized or palpable trans-orally, are best targeted with computed tomography (CT) scan guidance.

W.R. Ryan, M.D., F.A.C.S.
Division of Head and Neck Oncologic, Endocrine and Salivary Surgery, Department of Otolaryngology—Head and Neck Surgery, University of California-San Francisco, San Francisco, CA, USA
e-mail: William.Ryan@ucsf.edu

A. Sean Alemi, M.D.
Department of Otolaryngology—Head and Neck Surgery, University of California-San Francisco, San Francisco, CA, USA

A. van Zante, M.D., Ph.D.
Department of Pathology, University of California-San Francisco, San Francisco, CA, USA

M.B. Gillespie et al. (eds.), *Gland-Preserving Salivary Surgery*,
https://doi.org/10.1007/978-3-319-58335-8_3

Equipment

23- or 25-gauge needles
10 cc syringes
Aspirating gun or syringe holder
1% lidocaine
Alcohol swabs
Glass slides
95% methanol in Coplin jars
Gauze
Adhesive bandages
Ultrasound transducer and machine
If evaluation of the specimen is planned:
0.5% toluidine blue or Diff-Quik stains
Microscope

FNA Procedural Steps

1. Prepare local anesthetic and a 25- or 23-guage needle on a 10 cc syringe attached to a syringe holder.
2. In cases of palpable and superficial lesions, immobilize the nodule between the fingers.
3. Clean the skin with an alcohol swab.
4. Apply local anesthetic along the intended needle path. *Do not inject the target lesion as this diminishes yield and results in cellular artifact.*
5. Insert the biopsy needle through the skin into the nodule and then apply gentle suction (1–2 cc). *Excessive suction can result in hemodilution and diminished cellularity.*
6. Maintain suction, while the needle transverses the long axis of the nodule approximately ten times or until a trace of blood is detected in the needle hub.
7. Release suction and then withdraw the needle from the nodule and overlying skin.
8. An assistant (or the patient) should apply direct, firm pressure to the site.
9. Remove the needle from the syringe, aspirate 5 cc of air into the syringe, then reattach the needle, and expel the material onto glass slide(s).
10. Immediately smear slides and drop into alcohol fixative (for Papanicolaou stain) or allow to air-dry (for Diff-Quik stain). *Only a small droplet should be applied to each slide, and care should be taken to apply consistent pressure to distribute the material evenly.*

FNA technique should be rehearsed so that a sample can be expediently obtained, smeared, and fixed. Clotted specimens and/or thick smears can limit visualization of otherwise cellular aspirates. Fresh beef or chicken liver can be utilized to practice biopsy and smear techniques. Targeting nodules under ultrasound guidance should be rehearsed with an ultrasound phantom containing targets of various size and contour.

Immediate Assessment

Immediate microscopic assessment of FNA samples at the bedside is invaluable to ascertain whether the sample is sufficiently cellular for a diagnosis to be rendered. Evaluation can be performed on alcohol-fixed slides using toluidine blue (a temporary dye) or on air-dried slides stained with Diff-Quik. If the specimen is inadequate, the procedure should be repeated. When sampled appropriately, less than 10% of FNA specimens are insufficient for diagnosis. A recent publication of ultrasound-guided FNA for head and neck masses, including thyroid nodules, salivary gland masses, and lymph nodes, provides excellent tips as a practice guide [2]. In this study, 617 cases were reviewed, and only 6.1% of samples were insufficient for diagnosis. Samples are more likely to be nondiagnostic if the lesion is entirely cystic or if rim calcification is present. Dense, fibrous lesions generally yield scant material, and highly vascular targets can result in significant hemodilution of the sample. Overall, specimen quality increases in association with the experience of the clinician performing the biopsy, with a threshold of approximately 100 procedures [2]. The feedback from immediate assessment can result in improved biopsy technique and appropriate triage of the specimen for adjunctive studies.

Specimen Triage

If necessary, a formalin-fixed sample can be prepared by gently rinsing the contents of the needle and hub into a small volume of formalin with a syringe. Centrifugation of formalin-fixed material results in a “cell block” which can be

paraffin embedded. This type of preparation should be processed identically to a small punch or incisional biopsy and allows for special studies such as immunohistochemistry or fluorescence in situ hybridization (FISH) to be performed [3]. Labs have different methods of cell block processing. Careful attention to cellular yield, specimen preservation, and sectioning helps in achieve optimal histologic specimens. If lymphoma is suspected based on the clinical presentation and/or immediate assessment, a sample should be submitted for flow cytometric immunophenotyping. This study primarily provides information regarding the cell surface markers on lymphoid cells and can establish monoclonality, a finding that supports the diagnosis of hematologic malignancy. Specimens intended for flow cytometry should be held at room temperature in fresh cell culture medium. Samples for parathyroid hormone, thyroglobulin testing, or molecular studies require special handling, and instructions should be obtained from the clinical lab prior to the biopsy procedure.

Surgeon-Performed FNA

Surgeon-performed fine needle aspiration biopsy with or without ultrasound (US) guidance is certainly possible and can be particularly convenient for the patient. Separately scheduled biopsy procedures require additional communication and transportation and have the potential for delay in diagnosis and treatment. Surgeon-performed biopsy eliminates the possibility of miscommunication regarding the target(s) for biopsy. Collaboration between a surgeon who performs FNA and the cytopathologist who assesses for specimen adequacy can result in a preliminary/working diagnosis and expedite evaluation and treatment planning. Importantly, pathologists have variable training and experience in providing support in the clinical setting. At some institutions, cytotechnologists (technologists trained in interpretation of cytologic samples) are deployed to provide support, including specimen preparation, rapid interpretation, and triage. At some medical centers (including the authors' institution), cytopathologists are available to visit the surgeon's clinic and perform FNA biopsies. This practice has many advantages, including patient convenience, the opportunity for immediate communication of preliminary results, and frees up the surgeons to continue work in clinic with other patients, while the procedure is being performed.

Ultrasound-Guided FNA

Ultrasound-guided FNA (USGFNA) is important for non-palpable, ill-defined, or deep nodules. With appropriate training, many structures in the head and neck can be visualized in a safe and convenient manner utilizing clinical US. US guidance, particularly for the submandibular and parotid glands, may increase accuracy [4–6]. FNA of lesions of the sublingual gland and minor salivary glands are not likely to require ultrasound guidance, given that they are frequently accessible via a trans-oral approach.

Depending on the level of training and availability of experienced surgeons, cytopathologists, and radiologists at a particular institution, the optimal practitioner performing FNA and/or USGFNA may vary. Surgeon-performed ultrasound is popular in Europe and is gaining popularity in the United States. Similarly, surgeon-performed USGFNA procedures are becoming routine. One principle advantage of surgeon-performed USGFNA is the ability to correlate history and physical examination findings with US images and, in the optimal setting, preliminary cytology results within a single office visit. Other advantages of surgeon-performed biopsies include convenience to the patient, less potential for lapse in communication, and expedited workup. In many clinical settings, trained cytopathologists and/or radiologists are not immediately available to perform FNA; thus surgeons have the opportunity to gain expertise in US imaging and biopsy. Additionally, ultrasound-guided FNA can be useful in sampling some palpable lesions and can contribute to a higher diagnostic rate when compared to

standard palpation techniques alone [7]. However, these advantages need to be weighed against the need for additional training and continual practice to maintain expertise, as well as the potential impact on the surgeon's efficiency in the clinic.

CT-Guided FNA

CT-guided FNA is sometimes necessary for targeting salivary lesions arising from the deep lobe of the parotid gland and occupying the parapharyngeal space. Tumors arising from submucosal minor salivary glands along the upper aerodigestive tract may not be accessible transorally nor visible by ultrasound. Interventional radiologists typically perform CT-guided FNA after cross-sectional imaging is obtained. These procedures require significant time and resources and are, in general, performed under conscious sedation in addition to local anesthesia. The procedure for CT-guided FNA is similar to palpation-guided procedures; however a guiding needle may be placed and multiple specimens obtained through this "coaxial" system. Patients undergo repeated CT imaging in order to guide the radiologist and confirm the site for biopsy. Given the expense of CT-guided procedures, rapid microscopic interpretation for adequacy by a cytotechnologist or cytopathologist is highly recommended.

Patient Perspective of FNA

Because of the smaller gauge needle, many patients prefer FNA instead of core needle biopsy or an incisional biopsy. The likelihood of significant hematoma or infectious complications is dramatically less with a fine needle when compared with more invasive procedures. An additional benefit to the patient is the option for a biopsy during the same office visit as their surgical consultation. If a system is in place where a cytopathologist can expedite review of the sample, the clinician can counsel the patient on management options during the initial consultation.

Benefits of FNA Versus Surgical Biopsy

While FNA lacks the tissue architecture offered by larger core needle or open biopsies, the high diagnostic accuracy and increased patient tolerance make FNA the diagnostic procedure of choice for salivary gland neoplasms. Moreover, the risk for seeding the tumor into the needle tract or tissue distortion due to biopsy site changes is minimized with smaller bore needles > 20 gauge. In addition, FNA is unlikely to result in bleeding thereby making it unnecessary to stop anticoagulant medications. Though there are very few contraindications to FNA, in some cases incisional or excisional biopsies should be favored when considering the risks and the suspected pathologic process. Examples of neoplasms which can result in a falsely reassuring FNA results include lipomatous lesions that are concerning for liposarcoma, some T-cell lymphomas, and unusual histiocytic tumors such as Rosai-Dorfman. Sometimes an FNA will not be diagnostic or show benign cellular elements when other studies suggest a neoplastic process. In these cases, a more substantial sample is required prior to definitive therapy. However, in some cases, even a scant FNA sample is informative and can support conservative management. For example, a schwannoma is a benign nerve sheath tumor which typically yields very scant material on FNA. A sample containing a few, bland, spindled cells consistent with nerve sheath elements can, in the appropriate clinical setting, safely be followed when correlated with a benign clinical examination and appropriate imaging such as MRI.

Fine Needle Aspiration Versus Frozen Section

Although frozen section has limitations, it allows assessment of larger tissue samples and can demonstrate histologic features (i.e., invasion) that can support the diagnosis of malignancy in some cases where cytologic analysis cannot. One study reviewed 220 cases of parotid gland FNA and compared results of FNA biopsy with frozen section histology in 57 of those cases. Sensitivity, specificity, and

accuracy for FNA were found to be 86%, 92%, and 90%, respectively. In comparison, the sensitivity, specificity, and accuracy of the frozen sections were 77%, 100%, and 88%. In this study, frozen sections changed four FNA diagnoses from malignant to benign and clarified the diagnosis in 5 of 12 cases where FNA was nondiagnostic [8]. Thus, depending on the practice setting, FNA can be more sensitive, while frozen section can be more specific. Where both high-quality cytopathology and frozen section services are available, the two techniques are complementary.

Complications of FNA

FNA is generally considered to be safe; complications are extremely rare. Patients should be advised of these risks during the informed consent process prior to the procedure.

Inadequate Sampling

Inadequate sampling is biggest source of diagnostic error in cytopathology [9]. Rapid assessment can reduce the number of insufficient samples, but in the absence of immediate evaluation, nondiagnostic procedures or false-negative specimens should be expected. If the cytologic diagnosis is not in accord with the imaging findings or clinical impression, further evaluation should be pursued with consideration of repeat fine needle, core biopsy, or an excisional biopsy.

Anxiety and Discomfort

Most patients benefit from a clear explanation of the FNA procedure and the application of local anesthesia along the needle tract. Given that anesthetic should not be injected into the target lesion, many patients will have sharp, transient pain during and, in some cases, immediately after the biopsy procedure. Patients should be informed that some discomfort is expected but is of limited duration. Significant radiating pain can be associated with biopsy of a benign or malignant nerve sheath tumor or in cases of a malignancy with perineural invasion; the patient's report of significant radiating or lasting discomfort in these cases can be diagnostically informative.

Local Hemorrhage/Hematoma

Although bleeding at the insertion site and tract of the needle is certainly possible given the vascularity of the regions surrounding the salivary glands, this is unlikely to be a clinically significant or a common problem. Applying firm pressure in the site immediately after the biopsy can prevent and reduce hemorrhage and hematoma formation. A higher risk of hemorrhage or hematoma exists for patients on anticoagulant medications. In such patients, superficial nodules may be aspirated with minimal risk. For deeper nodules, or lesions in close proximity to larger caliber blood vessels, stopping anticoagulants prior to the procedure should be considered.

Infection

Infection from FNA biopsy is extremely rare and is closely correlated with the patient's immune status. The risk is equivalent to that of phlebotomy. The skin should be cleaned with an alcohol swab prior to biopsy, and sterile technique should be maintained during the procedure.

Syncope

Some patients are susceptible to vasovagal reactions to needle insertion. Performing aspiration while the patient is lying down or, at a minimum, sitting may help prevent this complication. All patients should be observed for several minutes prior to discharge from the clinical setting.

Needle Tract Contamination by Malignant Cells

Numerous studies indicate that needle tract contamination by malignant cells is a very rare complication with thousands of fine needle aspirations

performed worldwide yearly. A study of salivary gland adenomas found tumor cells along the needle track immediately following aspiration with a 22-gauge needle, but this was not shown to increase tumor recurrence at 5-year follow-up [10]. Theoretically, a risk of dissemination of dislodged neoplastic cells into lymphatics and blood vessels exists: *this risk appears to be lower in FNA than with incisional biopsy.* There was no seeding risk found in a study of 94 resected masses based on histopathologic assessment of specimens [11].

Fibrosis and Biopsy Site Changes

Despite being a relatively small needle, minor trauma caused by FNA can result in fibrosis or scarring around important structures, particularly around the facial nerve. This can create manual and visual difficulties during subsequent surgical dissection in the area. Furthermore, tumor infarction, displacement of neoplastic cells, and fibrosis can complicate pathologic assessment after FNA biopsy is performed preoperatively. Pathologists must take the history of biopsy into account when assessing invasiveness. Biopsy site changes can be erroneously interpreted as capsular invasion of a neoplasm or extranodal extension of a metastatic tumor. Thus, the history of prior biopsy should be conveyed to the surgical pathologist when a specimen is submitted.

Diagnostic Accuracy

There are many different patterns of inflammatory disease and dozens of benign and malignant salivary gland neoplasms. Furthermore, many of these conditions are very rare. Studies have demonstrated that the sensitivity of FNA for salivary gland neoplasia ranges from 80 to 100% while the specificity ranges from 90% to 100% [8, 12, 13]. In high-volume academic centers, salivary FNA has a positive predictive value of 80–98% [14–18] and can correctly differentiate between malignant and benign tumors 81–98% of the time [8]. These accuracy values are higher for benign neoplasms compared to malignancies [19, 20]. Higher accuracy is associated with more experienced cytopathologists, higher volume of specimens, and academic institutions compared to community practice settings [21, 22]. The level of expertise should be taken into account by the clinician when managing salivary pathology. Ultimately, inadequate sampling is biggest source of error [9]. Even with appropriate sampling, some patients may require excision for definitive diagnosis.

Possible Sources of Error in Salivary Gland FNA

Sampling Error

As previously mentioned, an insufficient sample is the most common error in salivary gland FNA. Direct communication between the surgeon (or clinician) and the cytopathologist to confirm the location of the proposed target can minimize such errors. Proper immobilization of the lesion additionally helps reduce under-sampling.

Interpretation Error

Errors in interpretation are inversely related to the experience of the cytopathologist; high-volume centers with more experienced pathologists will be less predisposed to such errors. Clinicians should consider requesting consultation with a cytopathologist with salivary expertise when the cytologic diagnosis is vague or at odds with the clinical presentation and the specimen is otherwise adequate.

Bias

The clinical setting can inform the cytologic diagnosis; thus it is advantageous for the same cytopathologist to perform the biopsy and interpret the specimen. However, pathologists can be biased based on past experience, the clinical picture, and/or the clinician's opinion. These factors can potentially lead to errors in diagnosis [23].

Technical Problems

Delay in fixation of smears can lead to air-drying artifact, one of the most common technical

Table 3.1 Common diagnostic considerations in FNA of salivary gland

Benign neoplasms	Malignant neoplasms	Lymphadenopathies	Inflammatory conditions	Cystic lesions
Pleomorphic adenoma	Mucoepidermoid carcinoma	Reactive lymph node	Acute sialadenitis	Branchial cleft cyst
Warthin tumor	Adenoid cystic carcinoma	Sarcoidosis	Chronic sialadenitis	Lymphoepithelial cyst
Basal cell adenoma	Basal cell adenocarcinoma	Lymphoma	Sjögren's syndrome	Mucous retention cyst
Myoepithelioma	Salivary duct carcinoma	Metastasis (carcinoma or melanoma)	IgG4-related sialadenitis	Cystic metastasis

problems in cytopathology. In addition, smears can be obscured by peripheral blood, fibrin stands/clot, inflammation, or ultrasound gel. Poor stain quality can limit cellular detail. These factors can result in false-positive or false-negative diagnoses.

Diagnostic Categories

There are five broad categories in salivary gland disease identified in cytologic specimens: normal, inflammatory/cystic masses, intraparotid lymphadenopathy, benign neoplasms, and malignant neoplasms. See Table 3.1 for common diagnostic considerations in FNA of salivary gland.

Normal Salivary Tissue

Given that a biopsy needle often traverses normal gland, normal salivary tissue can often be found even in abnormal samples (abnormal and normal tissue are mixed). Missing the target with the needle will also result in the finding of normal salivary elements. In lesions such as sialosis, hamartoma, or even lipoadenoma, samples contain only normal/expected tissues. Aspiration of a benign gland results in acinar and ductal cells admixed with adipose tissue. Normal lymphoid tissue is obtained when lymph nodes in or adjacent to the gland are aspirated. Serous and/or mucinous type acinar cells are found in various proportions with the parotid gland showing predominantly serous type, the submandibular gland showing serous and mucinous types, and mostly mucinous type in the minor salivary glands. Acinar cells are extremely delicate with pyramidal shape, granular or pale mucinous cytoplasm, and compact, round nuclei. Intact serous acinar cells are usually cohesive and found in grape-like clusters. Ductal cells have cuboidal or columnar shape with relatively dense cytoplasm and usually form tubules or honeycomb-like flat sheets. Adipose tissue generally consists of large lipid-filled cells with small, round, peripheral nuclei.

Inflammatory Conditions

Acute sialadenitis is typically a clinical diagnosis, and FNA is not generally indicated for patients with the expected clinical presentation. If frank pus is aspirated or immediate assessment of an FNA sample demonstrates abundant neutrophils and necrotic debris, material should be submitted for microbiologic cultures. Similarly, patients presenting with classic signs/symptoms of chronic sialadenitis do not require FNA. However some cases of focal duct obstruction or submandibular ptosis can mimic a neoplasm. Fine needle aspiration typically yields a heterogeneous population of lymphocytes admixed with scant atrophic ducts. Granular mineralized debris can be seen in cases of obstruction by sialolith(s). When abundant chronic inflammation and normal acini are lacking, FNA samples of chronic sialadenitis containing atrophic ductal epithelium can be misinterpreted as representing a "basal cell neoplasm." This is a known pitfall, but distinguishing benign atrophic ductal epithelium and neoplastic epithelium can be challenging, especially in the setting of prior radiation.

Both autoimmune sialadenitis and IgG4-related sialadenitis demonstrate cytologic features that overlap with non-specific/obstructive sialadenitis. Autoimmune etiology and a diagnosis of Sjögren's syndrome can be supported by incisional biopsy of labial salivary glands and appropriate serologic studies. The diagnostic features of IgG4-related disease have been established for histologic specimens and include a lymphoplasmacytic infiltrate with numerous IgG4+ plasma cells, storiform fibrosis, and obliterative phlebitis. Fibrosis and phlebitis are not evaluable in FNA specimens. Thus, if this diagnosis is a consideration, most clinicians would consider incisional biopsy. FNA criteria for IgG4-related disease have not been established; however if FNA biopsy is undertaken, a cell block should be prepared and immunostaining for IgG4+ plasma cells performed. Serum IgG4 is also frequently elevated in this condition and can be supportive and should be considered especially if there is evidence of multi-gland (e.g., liver, gallbladder, pancreas) involvement.

Cystic Lesions

Significant overlap exists between the cytologic features of various cystic lesions of the lateral neck. Careful evaluation of the epithelial lining of a cystic mass is essential to arrive at the correct diagnosis. Unfortunately, cyst fluid samples are typically dominated by proteinaceous fluid and histiocytes with degenerated lining cells representing a minor component. Targeting of the cyst wall under ultrasound guidance can sometimes be helpful, even when the mass is otherwise palpable. The possibility of cystic metastatic squamous cell carcinoma should always be considered in adult patients. Other diagnostic entities are branchial cleft cyst, lymphoepithelial cyst, mucocele/mucous retention cyst, and a cystic Warthin tumor. Developmental remnants are more likely in pediatric and young adult patients, and the identification of ciliated columnar "respiratory-type" epithelium, when present, is characteristic of a branchial cleft cyst.

Lymphoepithelial cysts show scant attenuated epithelium in a background of abundant reactive lymphoid tissue. These cysts tend to be bilateral, have characteristic imaging features, and are most common in patients with human immunodeficiency virus (HIV) infection. Typically, these patients can be managed by serial examinations and occasional therapeutic aspiration. While mucous extravasation most commonly involves the minor glands of the lip, a larger pseudocyst or ranula can arise from the sublingual or, less frequently, from the submandibular gland. This entity is exceedingly rare in the parotid region, and a mucoid aspirate from the parotid gland is most suggestive of a cystic mucoepidermoid carcinoma. Nonneoplastic mucinous lesions are usually hypocellular, with minimal atypia. The presence of abundant or atypical mucinous epithelium favors the diagnosis of a neoplasm. If FNA is equivocal, excision of the gland may be necessary for both diagnostic and therapeutic purposes.

Intraparotid Lymphadenopathy

The parotid gland contains a rich network of lymphatics that drain the auricle and scalp. These nodes can become enlarged as a result of inflammatory, benign, or malignant disease. Reactive lymph nodes can occur as a result of transient viral or bacterial infections and are rarely cause for concern. Persistent lymph node enlargement and abnormal radiographic appearance should trigger further evaluation. Metastatic squamous cell carcinoma from the scalp, auricle, or external auditory canal skin can present as nodal disease within the parotid. Squamous cell carcinoma is the most common metastasis to the parotid and much more likely than squamous cell carcinoma primary to the salivary gland. Melanoma also commonly metastasizes to the intra- or periparotid lymph nodes. Accordingly, patients with enlarged nodes should be questioned regarding a history of cutaneous malignancy.

While parotid enlargement can frequently indicate nodal metastasis, several systemic inflammatory conditions exist which can mimic

neoplastic processes. Patients with sarcoidosis can develop parotitis, uveitis, and fever, a condition known as Heerfordt's syndrome. Similarly, autoimmune destruction of salivary glands as seen in Sjögren's disease can cause gland enlargement. Sjögren's is associated with low-grade marginal zone (MALT) lymphoma. Thus, even when the clinical setting suggests an autoimmune process, FNA sampling may be indicated to exclude a lymphoproliferative disorder.

The diagnosis of lymphoma by FNA typically rests on a combination of morphology and immunophenotyping by flow cytometry. High-grade lymphoma generally consists of large, markedly abnormal lymphocytes. In contrast, low-grade lymphoma typically consists of monotonous, small, mature-appearing lymphocytes. Flow cytometric analysis can demonstrate monoclonality along with co-expression of characteristic cell surface markers allowing appropriate subtyping. However flow cytometry requires additional sampling and expedient processing. At times, an incisional or excisional biopsy may be necessary to obtain tissue architecture or for immunohistochemical stains for definitive classification of a hematopoietic neoplasm. In cases where FNA is suspicious, but a surgical biopsy is necessary to characterize a lymphoma involving intra- or peri-parotid lymph nodes, an open lymph node biopsy should be considered. Parotidectomy can generally be avoided in these cases, sparing the patient extensive surgery and potential morbidity. While clinical history and examination are helpful in differentiation of inflammatory and neoplastic lymphadenopathy, FNA can contribute significantly. The false-negative rate of lymph node FNA performed by expert cytopathologists is approximately 2–3%. Thus, most patients with a benign FNA can safely be followed.

Benign Salivary Neoplasms

The majority of salivary gland tumors are benign. Pleomorphic adenoma and Warthin tumor (papillary cystadenoma lymphomatosum) make up most of these benign neoplasms. Cytologic diagnosis of pleomorphic adenoma is generally straightforward; however usually cellular specimens or the presence of atypia may result in a less specific diagnosis of "low-grade neoplasm" being rendered. Smears characteristically show ductal and myoepithelial elements along with extracellular fibrillary stroma. When all three elements are present, the sensitivity and specificity of FNA for pleomorphic adenoma are extremely high [24]. Generally, given the risk of continued growth and malignant transformation, pleomorphic adenomas should be excised. Similarly, the recommendation is that basal cell adenoma and myoepithelioma be completely excised as basal cell adenoma can be confused with adenoid cystic carcinoma and histologic evaluation is required to definitively distinguish an adenoma or myoepithelioma and adenocarcinoma or myoepithelial carcinoma.

Warthin tumor, similar to pleomorphic adenoma, has characteristic cytologic findings. Aspirate smears show sheets of oncocytic epithelium associated with lymphocytes in a background of proteinaceous fluid. Findings are typically definitive; however infrequently squamous or mucinous metaplasia can be present and raise the concern for carcinoma. Warthin tumors most often arise in patients with a history of smoking. Thus, concern for malignancy based on atypical cytologic findings may prompt excision for diagnostic purposes. Less common benign salivary tumors include oncocytoma, sebaceous adenoma, and lymphadenoma. Depending on the degree of certainty of the cytologic diagnosis, observation can be implemented for these other benign neoplasms, especially in elderly patients or patients at higher risk for general anesthesia.

Benign FNA in the Context of a Suspicious Nodule

Sometimes, despite a benign cytologic diagnosis, clinical findings are sufficiently concerning that surgical excision should be considered. Suspicious findings include pain, infiltrative borders, and ipsilateral facial nerve palsy. FNA specimens should be acquired in a manner that attempts to sample different areas of a lesion. However technical factors or patient tolerance

sometimes limits sampling. Thus, a benign cytologic diagnosis should always be considered in the context of the clinical history and physical exam. Surgery should be undertaken when suspicious clinical findings persist. Finally, surgical resection is also reasonable if patients have a cosmetic concern, especially if surgical risk is low.

Malignant Salivary Neoplasms

The diagnosis of malignancy for some low-grade salivary gland tumors rests on the histologic finding of invasion. For this reason, neoplasms lacking marked cytologic atypia may be assigned a general diagnostic category such as "low-grade salivary gland neoplasm" or "basaloid neoplasm" with a differential diagnosis. This practice allows for surgical planning despite the limitations inherent to a cytologic sample. The most common salivary gland malignancy is mucoepidermoid carcinoma comprising approximately 10–15% of all salivary gland neoplasms and being found in all major and minor salivary glands [25]. The majority of mucoepidermoid carcinomas are low grade, and it is these predominantly cystic, low-grade tumors that complicate the assessment of mucinous cysts.

Similar to a pleomorphic adenoma, an FNA sample of an adenoid cystic carcinoma consists of compact "basaloid" epithelial and myoepithelial cells along with metachromatic stromal material. For this reason, aspirates that lack unequivocal features of adenoid cystic carcinoma, including cribriform architecture and sharply demarcated metachromatic hyaline stromal spheres and cylinders, are assigned a diagnosis of "basaloid" or "basal cell" neoplasm. The differential typically includes benign entities such as pleomorphic adenoma and basal cell adenoma along with malignancies such as basal cell adenocarcinoma and adenoid cystic carcinoma. Standard immunohistochemical stains cannot distinguish between these entities; however the majority of adenoid cystic carcinomas have a translocation that results in a fusion of the MYB and NFIB transcription factors [26, 27]. If available, positive immunohistochemical staining for MYB can support the diagnosis of adenoid cystic carcinoma.

Mammary analogue secretory carcinoma of the salivary gland similarly has a characteristic translocation. Immunohistochemical reagents may contribute to definitive preoperative diagnosis of these tumors in the future. However, this type of specialized reagents is not available at all institutions. Collecting FNA specimens into formalin for processing as a cell block can allow the specimen to be submitted to a reference laboratory for appropriate adjunctive testing based on the cytologic findings.

As previously mentioned, the most common high-grade neoplasm found within the salivary gland is metastatic squamous cell carcinoma. Metastatic tumors from the skin and upper aerodigestive tract are common and can usually be recognized based on clinical findings and without extensive immunohistochemical evaluation. Careful history taking is warranted for other patients when the preliminary diagnosis is high-grade adenocarcinoma. High-grade tumors of salivary gland origin such as salivary duct carcinoma can have overlapping features with malignancies such as breast, prostatic, and pancreatic adenocarcinoma. In these cases, the cytopathologist should be informed of any history of systemic malignancy, and imaging may be warranted prior to extensive surgery.

Conclusions

Fine needle aspiration (FNA) is a safe and an effective technique in the primary diagnosis and surveillance of patients with salivary gland pathology. FNA can be performed in the outpatient office setting, and FNA specimens are suitable for adjunctive testing such as culture, immunostaining, and flow cytometry. The quality of the diagnosis obtained by FNA depends on the skill of the clinician obtaining the sample and the experience of the pathologist in interpreting it. Thus, the role of FNA in a practice setting depends on the expertise on hand. In a high-volume center with experienced cytopathologists, FNA is very frequently adequate to make decisions regarding patient management including

supporting conservative/non-operative management in some cases and limiting or extending the extent of surgery.

References

1. Heller KS, Dubner S, Chess Q, Attie JN. Value of fine needle aspiration biopsy of salivary gland masses in clinical decision-making. Am J Surg. 1992;164(6):667–70.
2. Ahn D, Kim H, Sohn JH, Choi JH, Na KJ. Surgeon-performed ultrasound-guided fine-needle aspiration cytology of head and neck mass lesions: sampling adequacy and diagnostic accuracy. Ann Surg Oncol. 2015;22(4):1360–5.
3. Suen KC. Atlas and text of aspiration biopsy cytology. Baltimore, MD: Williams & Wilkins; 1990.
4. Feld R, Nazarian LN, Needleman L, Lev-Toaff AS, Segal SR, Rao VM, Bibbo M, Lowry LD. Clinical impact of sonographically guided biopsy of salivary gland masses and surrounding lymph nodes. Ear Nose Throat J. 1999;78(12):905,908–12.
5. Gritzmann N, Hollerweger A, Macheiner P, Rettenbacher T. Sonography of soft tissue masses of the neck. J Clin Ultrasound. 2002;30(6):356–73. Review
6. Ivanová S, Slobodníková J, Janská E, Jozefáková J. Fine needle aspiration biopsy in a diagnostic workup algorithm of salivary gland tumors. Neoplasma. 2003;50(2):144–7.
7. Robitschek J, Straub M, Wirtz E, Klem C, Sniezek J. Diagnostic efficacy of surgeon-performed ultrasound-guided fine needle aspiration: a randomized controlled trial. Otolaryngol Head Neck Surg. 2010;142(3):306–9.
8. Seethala RR, LiVolsi VA, Baloch ZW. Relative accuracy of fine-needle aspiration and frozen section in the diagnosis of lesions of the parotid gland. Head Neck. 2005;27(3):217–23.
9. MacLeod CB, Frable WJ. Fine-needle aspiration biopsy of the salivary gland: problem cases. Diagn Cytopathol. 1993;9(2):216–24. discussion 224-5.
10. Engzell P, Jakobsson Å, Sigurdson Å, Zajicek J. Aspiration biopsy of metastatic carcinoma in lymph nodes of the neck: a review of 1 101 consecutive cases. Acta Otolaryngol. 1971;72(1–6):138–47.
11. Mukunyadzi P, et al. Tissue effects of salivary gland fine-needle aspiration. Does this procedure preclude accurate histologic diagnosis? Am J Clin Pathol. 2000;114(5):741–5.
12. Stewart CJR, MacKenzie K, McGarry GW, Mowat A. Fine-needle aspiration cytology of salivary gland: a review of 341 cases. Diagn Cytopathol. 2000;22:139–46.
13. Mohammed Nur M, Murphy M. Adequacy and accuracy of salivary gland fine needle aspiration cytology. Ir J Med Sci. 2016;185(3):711–6.
14. Gajanin R, Djurdjević D, Usaj SK, Eri Z, Ljubojević V, Karalić M, Risović T. Reliability of fine needle aspiration and ex tempore biopsy in the diagnosis of salivary glands lesions. Vojnosanit Pregl. 2014;71(11):1018–25.
15. Tryggvason G, Gailey MP, Hulstein SL, et al. Accuracy of fine-needle aspiration and imaging in the preoperative workup of salivary gland mass lesions treated surgically. Laryngoscope. 2013;123(1):158–63.
16. Nguansangiam S, Jesdapatarakul S, Dhanarak N, Sosrisakorn K. Accuracy of fine needle aspiration cytology of salivary gland lesions: routine diagnostic experience in Bangkok, Thailand. Asian Pac J Cancer Prev. 2012;13(4):1583–8.
17. Kechagias N, Ntomouchtsis A, Valeri R, et al. Fine-needle aspiration cytology of salivary gland tumours: a 10-year retrospective analysis. Oral Maxillofac Surg. 2012;16(1):35–40.
18. Siewert B, Kruskal JB, Kelly D, Sosna J, Kane RA. Utility and safety of ultrasound-guided fine-needle aspiration of salivary gland masses including a cytologist's review. J Ultrasound Med. 2004;23(6):777–83.
19. Oka K, Chikamatsu K, Eura M, Katsura F, Yumoto E, Tokunaga H. Clinical significance of fine-needle aspiration biopsy in major salivary gland tumors. Nihon Jibiinkoka Gakkai Kaiho. 2002;105(11):1109–15. Japanese
20. Verma K, Kapila K. Role of fine needle aspiration cytology in diagnosis of pleomorphic adenomas. Cytopathology. 2002;13(2):121–7.
21. Jandu M, Webster K. The role of operator experience in fine needle aspiration cytology of head and neck masses. Int J Oral Maxillofac Surg. 1999;28(6):441–4.
22. Pitts DB, Hilsinger RL Jr, Karandy E, Ross JC, Caro JE. Fine-needle aspiration in the diagnosis of salivary gland disorders in the community hospital setting. Arch Otolaryngol Head Neck Surg. 1992;118(5):479–82.
23. Schmidt RL, Jedrzkiewicz JD, Allred RJ, Matsuoka S, Witt BL. Verification bias in diagnostic accuracy studies for fine and core needle biopsy of salivary gland lesions in otolaryngology journals: a systematic review. Head Neck. 2014;36(11):1654–61.
24. Heaton CM, Chazen JL, van Zante A, Glastonbury CM, Kezirian EJ, Eisele DW. Pleomorphic adenoma of the major salivary glands: diagnostic utility of FNAB and MRI. Laryngoscope. 2013;123(12):3056–60.
25. Majewski J, Schwartzentruber J, Lalonde E, Montpetit A, Jabado N. What can exome sequencing do for you? J Med Genet. 2011;48(9):580–9.
26. The UK10K project. http://www.uk10k.org/. Accessed 25 Aug 2014.
27. Ding LE, Burnett L, Chesher D. The impact of reporting incidental findings from exome and whole-genome sequencing: predicted frequencies based on modeling. Genet Med. 2014;17(3):197–204.