Jointly sponsored by the University of Wisconsin School of Medicine and Public Health, Office of Continuing Professional Development in Medicine and Public Health and the Wisconsin State Laboratory of Hygiene.

Statement of Need: To reinforce the diagnostic features of medullary carcinoma and to reacquaint the reader with the clinical laboratory tests needed to support this diagnosis.

Target Audience: Cytopathologists, cytopathology fellows, and other healthcare professionals.

Learning Objectives: After completing this exercise, participants should be able to:

1. Identify the general features of medullary carcinoma of the thyroid.
2. Describe the cytologic morphology of medullary carcinoma of the thyroid derived from Fine Needle Aspiration samples.
3. Explain concepts influencing the diagnostic accuracy of Fine Needle Aspiration regarding medullary carcinoma of the thyroid and be aware of useful ancillary tests.
4. Describe the usual clinical course and therapy for medullary carcinoma of the thyroid.
5. Recall the genetics of medullary carcinoma of the thyroid and current information regarding sporadic cases and the association of the disease with the classical MEN syndromes.

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Method of Participation: This CME activity should take one hour to complete. Approximately 45 minutes should be spent reviewing the article. The remaining 15 minutes should be used to review and complete the posttest and evaluation. There are no prerequisites for participants.

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Acinic Cell Carcinoma of the Salivary Gland: A Continuing Medical Education Case

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Background

Head and neck malignancies account for ~6.6% of cancer registry diagnoses.¹ The most common head and neck neoplasms are squamous cell carcinoma, thyroid neoplasms, and those involving cervical lymph nodes (lymphoma and metastases). The salivary glands serve as additional infrequent sites for neoplasia, contributing 4.5% of head and neck (<1% of all) cancer diagnoses.² Anatomically, salivary gland tissue is comprised of both major and minor salivary glands. The major salivary glands include the bilateral parotid, submandibular, and sublingual glands. The major salivary glands are composed of seromucinous acini arranged in lobules around draining ducts. The parotid gland is primarily serous whereas the sublingual gland is predominantly mucinous. The submandibular gland demonstrates a mixture of cell types. Minor unnamed salivary glands are present in sinonasal, oropharyngeal, laryngeal, and bronchial mucosa. Although salivary gland neoplasms are uncommon, the superficial anatomy of the head and neck region makes this site easily amenable to fine-needle aspiration (FNA). This technique can supply important information to a clinician for patient management. FNA may be utilized to categorize a lesion as inflammatory or neoplastic, as lymphoid or epithelial, in addition to providing a specific diagnosis. Thus, in spite of their rarity, primary salivary gland neoplasms may be encountered in the “routine” cytologic practice because of clinical accessibility.

Several recent studies have examined the utility of FNA in the diagnosis of salivary gland lesions. In general, cytologic diagnoses demonstrate a high degree of sensitivity (70%–98%) and specificity (86%–100%).² Moreover, Seethala et al. examined 220 cases and showed FNA to have comparable sensitivity (86% versus 77%), specificity (92% versus 100%) and accuracy (90% versus 88%) to frozen section analysis (statistics for FNA and frozen section listed respectively).³ Because these techniques examine different histologic parameters (cytologic detail versus architecture), they may be viewed as complementary rather than mutually exclusive. Nonetheless, studies highlight potential pitfalls in the cytologic diagnosis of salivary gland lesions. Acinic cell carcinoma is recognized as one lesion which challenges diagnostic acumen.⁴ The difficulty in diagnosing acinic cell carcinoma contributed to five false-negative diagnoses in several case series with a total of 365 salivary gland lesions.⁵⁻⁷

Clinical Features

Acinic cell carcinoma is an uncommon malignant neoplasm of the salivary gland, accounting for <5% of all salivary gland neoplasms.⁸ Although generally considered a low grade lesion, malignancy and potential for metastasis was first recognized in the 1950s.⁹ This malignancy commonly arises in the parotid gland (~80%–90% of cases) with the minor salivary glands being the next most prevalent site (9%). Acinic cell carcinoma typically presents as a slowly growing painless or painful mass (Fig. C-1A). Occasionally, facial nerve paresis is a presenting symptom. Acinic cell carcinoma is also the most common bilateral primary salivary gland malignancy. There is a slight female predominance in cases. Although acinic cell carcinoma afflicts both adults and children, it is more prevalent among adults with highest incidence in the fifth and sixth decades.⁸,¹⁰,¹¹ It is the second most common salivary gland cancer in children after mucoepidermoid carcinoma, accounting for 3.4% of 118 pediatric parotid lesions in a 27-year series.¹²
Pathologic Features
The gross specimen of acinic cell carcinoma usually demonstrates a circumscribed nodular mass, although cystic degeneration may be observed. Microscopically, however, encapsulation is incomplete and areas of invasion are usually noted. Recurrent tumors may be multinodular/multifocal. Histologically, acinic cell carcinoma is characterized by serous acinar differentiation. The neoplasm is comprised of cells with polygonal borders and eccentric rounded nuclei polarized toward a secretory lumen. The finely granular cytoplasm contains basophilic zymogen secretory granules (Fig. C-1C).<sup>8,11,13</sup> Although serous acinar differentiation is predominant and defines this neoplasm, a variety of different cells types may be noted. These include intercalated duct cells (cuboidal cells with eosinophilic cytoplasm), nonspecific glandular cells (amphophilic cells with indistinct borders), and vacuolated cells to clear cells (cells with cytoplasm partially to completely filled by intracytoplasmic clear vacuoles). In part because of this diversity, the neoplastic cells are thought to originate from pluripotent reserve cells residing at the intercalated duct. The cellular diversity is thought to recapitulate differentiation of the pluripotent reserve cell toward serous acinar morphology.<sup>13,14</sup> The neoplastic cells of acinic cell carcinoma can be arranged in several morphologic patterns, including microcystic, solid, papillary cystic, and follicular (Fig. C-1E). Generally, tumors display both a mixture of types and histologic patterns, the specifics of which have no bearing upon prognosis.<sup>8,11</sup> Although histologic grading of acinic cell carcinomas remains controversial, these neoplasms can be divided based upon their degree of differentiation and nuclear features into low and high grades. The majority (>80%) of tumors are low grade (well to moderately differentiated).<sup>10</sup>

The cytologic features of acinic cell carcinoma were initially described by Eneroth in 1971 and have more recently been discussed by Nagel et al.<sup>15,16</sup> Aspirates of acinic cell carcinoma are highly cellular. They are characterized by a monomorphic population resembling serous acinic cells (Fig. C-1B). The neoplastic cells have slightly enlarged, round nuclei that are eccentrically located. The nuclear chromatin is evenly distributed chromatin with some conspicuous nucleoli. There is mild nuclear pleomorphism with occasional nuclear atypia. Acinic carcinoma cells have abundant cytoplasm with coarse zymogen granules, which are basophilic on Papanicolaou stain and eosinophilic on Diff-Quik stain. Sometimes, the cytoplasm can be degranulated, appearing foamy, vacuolated or clear. Not uncommonly, nuclei are stripped of cytoplasm, leaving many bare nuclei in the aspirate. The variety of cell types observed in surgical specimens is not typically appreciated. There is also generally an absence of ductal epithelium, stroma, and adipose tissue. Well-formed acinar structures like rosettes or micro-follicles can be appreciated in many instances. Other patterns such as trabecular and papillary morphology could be seen.

Ancillary Studies
Although ancillary studies are not entirely specific for acinic cell carcinoma, they may be helpful in supporting a diagnosis. The secretory granules of acinic cell carcinoma stain with periodic acid-Schiff and resist diastase digestion (Fig. C-1D). This staining pattern is particularly helpful in characterizing the cytoplasmic granularity of this neoplasm. Although seeing progressively less use in tumor diagnosis, electron microscopy can be useful in characterizing the zymogen granules and thus the neoplastic cells of acinic cell carcinoma. It also delineates the abundant rough endoplasmic reticulum, and mitochondria.<sup>14</sup> The immunoprofile, however, is nonspecific with neoplastic cells expressing cytokeratin, and zymogen granule components transferrin, lactoferrin, alpha-1-antitrypsin, alpha-1-antichymotrypsin, and amylase.<sup>13</sup>

Differential Diagnosis
In well differentiated acinic cell carcinoma, the tumor cells may be difficult to distinguish from normal serous acinar cells, which may be seen in a variety of benign conditions. For example, sialadenosis is a non-inflammatory process that often presents as bilateral swelling of the salivary glands, especially the parotids. Sialadenosis describes hyperplasia of the acinar cells; the specimen is cellular with rare ductal cells. Like acinic cell carcinoma, these benign acinar cells tend to have abundant cytoplasm and basally located nuclei; however, the nuclei are smaller with normal nuclear/cytoplasmic ratio in comparison to the acinic cell carcinoma. Although it is usually idiopathic, sialadenosis can be associated with systemic diseases such as diabetes, malnutrition, and alcoholism. Hence, clinical information can assist in making the diagnosis. In chronic sialadenitis, another benign salivary lesion, the aspirate displays scant cellularity unlike aspirates of acinic cell carcinoma. In contrast to the monomorphic population in aspirates from acinic cell carcinoma, in sialadenitis the population is polymorphic—ductal cells are present and usually outnumber acinar cells (Fig. C-2A). In sialadenitis the fewer acinar cells can form lobulated clusters around ductal epithelium resembling grapes. Moreover, stromal elements as adipose can be intermixed unlike in acinic cell carcinoma.

The differential diagnosis also includes oncocytic neoplasms. First, oncocytoma is a consideration. Oncocytoma is a rare benign salivary gland neoplasm that typically occurs in the parotid gland. It accounts for ~ 1% of salivary gland neoplasms. Oncocytomas are almost exclusively composed of oncocyes with abundant granular eosinophilic cytoplasm (Fig. C-2B). In comparison, the
Fig. C-1. CME Case Patient History. The patient is a 71-year-old female who had left-sided serous otitis media. After unsuccessful treatment with antibiotics, she presented to an otolaryngologist. Physical exam revealed a 5-cm parapharyngeal space mass displacing her oral cavity structures and oropharynx. CT scan (A) demonstrated a nonenhancing 5 × 3 × 4.5 cm heterogeneous mass in the left parapharyngeal space extending toward the left masticator space and into the carotid space. It occluded the left eustachian tube and extended superiorly toward the skull base. It appeared to arise from the deep portion of the left parotid gland. The radiologic features were considered most consistent with a benign mixed tumor, with other benign and malignant masses less-likely differential diagnostic possibilities. FNA was performed by clinician and material submitted was used to make a Thin-Prep Papanicolaou stained slide (B, 400× magnification) and a cell block (C, 400× magnification). In this case, upon realizing that the lesion could originate from the salivary gland, the typical features acinic cell carcinoma are appreciated. At low magnification, a moderately cellular, discohesive, monomorphic population of small cells is seen. Some cells form small clusters while others are individually scattered. No glandular structures are observed. The only other cells identified in the background are inflammatory and red blood cells. At higher magnification most lesional cells show distinct cell membranes. Nuclei are round and eccentrically located, with evenly distributed chromatin and small nucleoli. The nuclear/cytoplasm ratio is not significantly increased. The cytoplasm exhibits coarse granules. The cell block depicts a very cellular specimen with a rather uniform cell population forming sheets, small trabeculae, and even some micropapillae. Periodic acid-Schiff stain with diastase digestion is positive, demonstrating a red granular pattern to confirm the diagnosis (D, ×400 magnification). Cytologic findings recapitulate the features seen upon resection of acinic cell carcinoma (E, ×400 magnification). The tissue specimen exhibits enlarged cells with eccentric nuclei, fine chromatin, and pinpoint nucleoli arranged in a microcystic/follicular pattern. The basophilic zymogen granules are readily apparent.
cytoplasmic granularity in an oncocytoma is finer than that of acinic cell carcinoma. The granularity in oncocyto-
toma results from innumerable mitochondria rather than secretory granules as in acinic cell carcinoma. Moreover, oncocytoma does not have cytoplasmic vacuoles which may be present in acinic cell carcinoma. Periodic acid-
Schiff diastase staining or electron microscopy can assist in characterizing the granularity by delineating either the mitochondria of oncocytoma or the secretory granules of acinic cell carcinoma. Phosphotungstic acid hematoxylin is an additional histochemical stain which may be used to reveal mitochondria of an oncocytoma.

Warthin’s tumor, the second most common salivary gland neoplasm following pleomorphic adenoma, is another oncocytic neoplasm in the differential diagnosis with acinic cell carcinoma. Warthin’s tumor typically has large oncocytic cells with a single, round nucleus with a prominent nucleolus. These are arranged as sheets of columnar epithelium. In some instances, as in ThinPrep cyto-
tology, the cells may appear more rounded and hence mimic acinic carcinoma cells. The epithelial cells of Warthin’s tumor are accompanied by a polymorphous lymphoid stroma. Occasionally, the bare nuclei of acinic cell carcinoma mimic lymphocytes of Warthin’s tumor, although lymphocytes are smaller with coarser nuclear chromatin and with a rim of remaining cytoplasm. This lymphocyte population and degenerative cystic material are commonly considered important clues for the diagno-
sis of Warthin’s tumor. Of note, though, other salivary gland neoplasms may be accompanied by a lymphoid

**Fig. C-2. Differential Diagnostic Considerations.** Chronic sialadenitis displays a polymorphic population with benign ductal cells (right) and acinar cells (left) in grape-like clusters (A, ThinPrep Papanicolaou stained slide ×400 magnification). Oncocytoma shows a monomorphic population of cells with eccentric nuclei, finely granular cytoplasm, and lack of vacuoles (B, Papanicolaou stained slide ×400 magnification). Salivary duct carcinoma is a high-grade malignancy exhibiting enlarged, irregular nuclei with prominent nucleoli (C, ThinPrep Papanicolaou stained slide ×400 magnification). Met-
astatic clear cell renal carcinoma also shows high-grade features of nuclear pleomorphism, increased nuclear-cytoplasmic ratios, irregular contours and prominent nucleoli in cells with finely vacuolated/flocculent cytoplasm (D, Papanicolaou stained slide ×400 magnification). In addition to history of a renal mass, positive immunostaining with CD10 or RCC-Ma may aid in this diagnosis.
stroma. A lymphoid stroma has been described for a subset of acinic cell carcinomas, as well, further complicating this differential diagnosis.\textsuperscript{16,17} Recently, cytologic features of acinic cell carcinoma with papillary cystic histology have been discussed. This is a diagnostic challenge since the classic features of acinic cell carcinoma are muted (with few naked tumor nuclei, increased nuclear/cytoplasmic ratios and cells grouped in a papillary rather than acinar architecture, and the distracter of cyst contents with macrophages and debris).\textsuperscript{18} Thus, careful discrimination of the above described features of the nonlymphoid epithelial provides the key to the diagnosis.

Granular cell tumor is a third potentially oncocytic mimic of acinic cell carcinoma. Granular cell tumors are generally mucosally based. They display aggregates and discohesive cells on aspiration. The polygonal cells are large with acidophilic granular cytoplasm and polarized pale nuclei with prominent nucleoli. If this enters the differential diagnosis, immunostaining for S100, which is positive in granular cell tumors, can be of assistance.

A high-grade (poorly differentiated or undifferentiated) acinic cell carcinoma is more difficult to recognize and is likely be classified on FNA as adenocarcinoma, not otherwise specified. For this reason, other high-grade salivary gland neoplasms such ductal carcinoma and high-grade mucoepidermal carcinoma enter the differential diagnosis. Salivary duct carcinoma aspirates are highly cellular with discohesive atypical cells and background necrosis (Fig. C-2C). It may be distinguished by positive immunostaining with androgen receptor. Although the mucin-secreting cells of mucoepidermoid carcinoma may mimic vacuolated cells of acinic cell carcinoma, mucoepidermoid carcinomas generally have a variation in cell types with intermediate and squamous cells with denser cytoplasm also present. Additionally, in the setting of a high-grade malignant neoplasm, other malignancies such as lymphoma, metastatic carcinoma (Hurthle cell or renal cell, Fig. C-2D), and melanoma are in the differential diagnosis list. With these diagnostic situations, the immunoprofile and clinical history are useful.

**Treatment/Prognosis**

Acinic cell carcinomas are usually considered low-grade malignancies. The 5 year disease-specific survival averages 91\%\textsuperscript{10} with a longer term, lifetime disease-associated death incidence of 16\%.\textsuperscript{13} Nonetheless, local recurrence is not uncommon, occurring in about 28\%–35\% of patients.\textsuperscript{10,13} Metastasis occurs in \(\sim 7\%–16\%\) of patients and most frequently involves cervical lymph nodes before more distant hematogenous sites.\textsuperscript{10,13} Acinic cell carcinoma can have a protracted clinical course with recurrence and metastasis even decades after the initial diagnosis. Surgical therapy is the primary treatment for acinic cell carcinoma. This is generally comprised of partial or total parotidectomy. If aggressive features such as high histologic grade, elevated stage, positive surgical margins, or lymph node metastases are identified more aggressive therapy may be recommended. This may consist of adjuvant neck dissection and regional radiotherapy.

**Conclusions**

In summary, although the diagnosis of salivary gland neoplasms in general and acinic cell carcinoma in particular can seem daunting, a diagnostic algorithm can help achieve insight. First, where is the lesion (oropharyngeal mucosa, soft tissue, lymph node, or salivary gland)? Second, is the process neoplastic? Third, what are the characteristics of the cells and or stroma? This question supplies information to determine whether the process is benign or malignant, and primary or metastatic. Such an approach can assist in consideration of the differential diagnoses described above and allow recognition of neoplastic acinar cells. Then a diagnosis of acinic cell carcinoma to be made with increased confidence.

**References**


Learning Objectives: After completing this exercise, participants should be able to:

1. Identify the general features of acinic cell carcinoma of the salivary gland.
2. Describe the cytologic morphology of acinic cell carcinoma of the salivary gland derived from Fine Needle Aspiration samples.
3. Consider the differential diagnosis of acinic cell carcinoma.
4. Explain concepts influencing the diagnostic accuracy of Fine Needle Aspiration regarding acinic cell carcinoma of the salivary gland and be aware of useful ancillary tests.
5. Describe the usual clinical course and therapy for acinic cell carcinoma of the salivary gland.

CME POSTTEST

Case of Acinic Cell Carcinoma of Salivary Gland, May 2008

True or False:

1. Estrogen receptor and pancytokeratin are useful immunostains for distinguishing acinic cell carcinoma from salivary duct carcinoma.
2. Helpful immunostains for distinguishing acinic cell carcinoma from melanoma include S100, melanA, and cytokeratin.
3. Salivary glands are an infrequent site for head and neck neoplasia, contributing <5% of cancer diagnoses.

Multiple Choice:

4. What is the role of fine needle aspiration in the diagnosis of salivary gland lesions?
   A. Fine needle aspiration plays no role; tissue biopsy is required for diagnosis.
   B. Fine needle aspiration is usefully primarily as a screening test, distinguishing benign from malignant.
   C. Fine needle aspiration is helpful, but results in seeding of needle tracts, which must later be excised.
   D. Fine needle aspiration is similar to frozen sections in terms of sensitivity, specificity, and diagnostic accuracy.

5. The most prevalent site for acinic cell carcinoma is (are) the ________ salivary gland(s).
   A. Parotid
   B. Sublingual
   C. Submandibular
   D. Minor

6. The majority of acinic cell carcinomas are
   A. Low grade
   B. Uncertain Malignant Potential
   C. High Grade
   D. Locally recurrent

7. Acinic cell carcinoma is most common in what age group?
   A. Children/adolescents
   B. 3rd–4th decades
   C. 5th–6th decades
   D. Greater than 6 decades

8. Which of the following is a (are) feature(s) of sialadenosis? (select all that apply)
   A. Acinar cell hyperplasia
   B. Bilateral
   C. Association with systemic disease (as diabetes)
   D. All of the above

9. Which feature(s) is (are) useful in distinguishing benign acinar cells of chronic sialadenitis from acinic cell carcinoma? (select all that apply)
   A. Scant cellularity of sialadenitis
   B. Admixture of stromal elements in sialadenitis
   C. Polymorphic cell population in sialadenitis with acinar and ductal cells
   D. All of the above

10. Which feature(s) is (are) useful in distinguishing oncocytoma from acinic cell carcinoma? (select all that apply)
    A. Nature of the cytoplasmic granules (size and electron microscopic characterization)
    B. Presence of cytoplasmic vacuoles in acinic cell carcinoma and their absence in oncocytoma
    C. Positive staining for phosphotungstic acid hematoxylin in oncocytoma and periodic acid-Schiff in acinic cell carcinoma
    D. All of the above

11. The primary treatment modality for low grade acinic cell carcinoma is ________.
    A. Surgical resection
    B. Surgical resection plus radiation therapy
    C. Radiation therapy
    D. Chemotherapy
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1. Overall, how do you rate the information presented in this publication?
   □ Excellent □ Very Good □ Good □ Fair □ Poor □ N/A

2. Please rate your ability to meet the following objectives, based on the content of this article:
   A. Identify the general features of acinic cell carcinoma of the salivary gland.
      □ Yes □ No
   B. Describe the cytologic morphology of acinic cell carcinoma of the salivary gland derived from Fine Needle Aspiration samples.
      □ Yes □ No
   C. Explain concepts influencing the diagnostic accuracy of Fine Needle Aspiration regarding acinic cell carcinoma of the salivary gland and be aware of useful ancillary tests.
      □ Yes □ No
   D. Describe the usual clinical course and therapy for acinic cell carcinoma of the salivary gland.
      □ Yes □ No

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These questions pertain to article, “Acinic Cell Carcinoma of the Salivary Gland: A Continuing Medical Education Case,” by Pu RT and Hall DA, on the previous pages.