SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 32: 237, 2026

Role of fine-needle aspiration cytology and cell morphology in determining the histogenesis of salivary gland lesions

BISHARAH SOUDAH[1] and MAHMOUD ABBAS[2]

[1]Institute of Pathology, Hannover Medical School, D-30625 Hannover, Germany;
[2]Gerhard-Domagk-Institute of Pathology, University Hospital Münster, D-48149 Münster, Germany

Received August 21, 2025; Accepted April 15, 2026

DOI: 10.3892/etm.2026.13233

Abstract. Fine-needle aspiration (FNA) of suspicious salivary and neck swellings is a simple and cost-effective diagnostic method for the evaluation of inflammatory and neoplastic conditions. The aim of the present study was to evaluate the diagnostic accuracy of FNA cytology (FNAC) in determining the histogenesis of salivary gland lesions based on cytomorphological features and associated histopathological outcomes to refine diagnostic criteria and contribute to improved diagnostic accuracy in salivary gland cytopathology. FNA was performed in 544 patients at the Institute of Pathology, Hannover Medical School (Hannover, Germany). The cytological smears were stained with May-Grunwald Giemsa, Papanicolaou and periodic acid-Schiff and compared with histological specimens from the same patients using immunohistochemical and molecular methods. The samples were anonymized and analyzed retrospectively. Of the total cases, 422 (77.57%) were benign lesions, 59 (10.85%) were malignant lesions and 26 (4.78%) were normal cases. Insufficient material was obtained in 37/544 cases (6.80%). No complications were observed. The concordance with histological findings for neoplastic lesions was >97.5%, with a false negative rate of 1.7%. The sensitivity of the diagnostic methods was 94% and the specificity was 98%. Of the 544 cases, 283 were female (52%) and 261 were male (48%). The diagnostic value of FNAC in representative material was relatively high in distinguishing between benign and malignant lesions. Sensitivity and specificity were variable (60.0 -97.5%). Correct diagnosis in the head and neck region requires interdisciplinary integration of findings and pathological-clinical cooperation.

Correspondence to: Dr Mahmoud Abbas, Gerhard-Domagk-Institute of Pathology, University Hospital Münster, Domagkstrasse 17, D-48149 Münster, Germany
E-mail: mahmoud.abbas@ukmuenster.de

Key words: fine needle aspiration cytology, salivary gland tumor, morphological and diagnostic hallmark

Introduction

Salivary gland tumors account for ~3% of all head and neck neoplasms, the vast majority (>90%) of which are of epithelial origin (1). Despite advances in imaging techniques, no single modality provides reliable differentiation between benign and malignant salivary gland lesions. Although features such as irregular tumor margins may suggest malignancy, imaging findings alone are typically insufficient for definitive diagnosis (2).

Fine-needle aspiration cytology (FNAC) is a minimally invasive diagnostic tool in the evaluation of salivary gland lesions, used due to the superficial location and accessibility of these glands (3). FNAC serves a key role in distinguishing between inflammatory, cystic and neoplastic processes and contributes to preoperative decision-making. However, its diagnostic accuracy depends not only on sampling quality and cytopathological expertise but also on the integration of clinical findings, patient history and anatomical localization (4-7).

A challenge in salivary gland cytology lies in the marked morphological diversity of both benign and malignant lesions, as well as the overlapping cytological features between different tumor entities. This heterogeneity complicates not only the differentiation between benign and malignant processes but also the determination of tumor histogenesis. While characteristic cytomorphological patterns have been described for numerous entities, their interpretation requires experience and remains prone to diagnostic pitfalls (8).

In recent years, molecular pathological findings have increasingly contributed to the classification and diagnosis of salivary gland tumors, particularly in malignant cases. Nevertheless, cytomorphology remains the cornerstone of initial diagnostic assessment, especially in routine clinical practice where molecular testing is not always readily available or feasible. Given these challenges, a comprehensive evaluation of the diagnostic value of FNAC, particularly with regard to cytomorphological criteria and their role in determining histogenesis, is of considerable clinical relevance (9).

There are three major salivary glands: Parotid, the sublingual and the submandibular glands. The parotid gland, a serous gland, is composed of acini, intercalated ducts, striated ducts and larger excretory ducts. It contains flat to highly prismatic epithelium, basal cells, myoepithelial cells, fat cells and connective tissue rich in collagen fibres (10).

Acute sialadenitis may be caused by bacteria, viruses (mumps), stasis, calculus or poor oral hygiene. In FNAC specimens, it may show inflammatory cells such as granulocytes and histiocytes, which can overlap with other inflammatory or neoplastic processes, making cytological interpretation challenging (10)

Chronic sialadenitis is characterized by the presence of numerous lymphocytes, plasma cells and fibroblasts. Epithelioid cell granulomas may be seen in diseases such as tuberculosis or sarcoidosis. Other causes of inflammation include Sjögren's syndrome, Heerfordt's syndrome and radiation or radioiodine therapy. Sjögren's syndrome manifests as notable atrophy of the parotid gland with signs of lymphocytic inflammation. Several small and partly budding globules of connective tissue are seen, surrounded by uniformly sized epithelial cells with normal-appearing nuclei. In the background there is blood and normal lymphocytes. Because Sjögren's syndrome is rare and can be diagnosed without FNA based on the patient history and associated clinical symptoms such as dry mouth and eyes, there are few descriptions of the cytological findings. An important feature is the proliferation of lymphocytes, which is observed in ~20% of FNA from the salivary glands (10,11).

A total of ~20% of all salivary gland lesions is cystic, and cystic components are often seen in local recurrence or metastases (10). Accurate classification of cysts can be challenging due to the wide spectrum of underlying benign and malignant entities, frequent cystic degeneration of solid tumors, and variable cellularity of aspirates, which may result in nondiagnostic or misleading cytological findings that can be challenging (12). Cytologically, fluid is seen in the background of the specimen with intercrystalline spaces. In addition, scattered inflammatory cells are typically present, along with possible evidence of previous hemorrhage or pathogens (12).

Pleomorphic adenoma (PA) is a benign tumor that most commonly occurs in the parotid gland, but can also be found in other salivary glands. It is the most common benign neoplasm of the salivary glands, accounting for 85% of cases and approximately half of all salivary gland tumors. The peak incidence is in the 4th to 6th decade of life, with a reported age range of 10-80 years and with a mean age of 43 years. Females are slightly more affected than males (3:2) (11,13). Cytologically, the tumor presents with a pink myxoid ground substance and characteristic stromal fissures. Chondroid cells, myoepithelial squamous metaplasia and columnar epithelial cells may also be present, forming isomorphic cell clusters. In addition, there are scattered cells with round nuclei and moderately wide cyanophilic cytoplasm. Identification of epithelial and myoepithelial/stoma components is key for the diagnosis of PA (12,14). PA has a tendency to recur, and in ~5% of cases, carcinoma may develop at an advanced age. Molecular genetic studies show a translocation at 8q12 and 12q14-15 in 70% of cases. Although PA is usually readily recognizable, its morphological heterogeneity may result in diagnostic challenges. Differential diagnostic considerations include carcinomas, myoepithelial neoplasms, and basal cell adenoma or carcinoma (11,13).

First described in 1929, Warthin's tumor (WT) is also known as cystadenolymphoma. The peak incidence occurs in the 6th to 7th decade of life (age range of 40-90 years). WT is typically found in the parotid gland (>90% of cases), although bilateral involvement may occur. Cytologically, WT presents with abundant lymphocytes with chromatin segregation, oncocytic cell clusters and occasionally cystic structures with crystal lacunae, macrophages and cell debris. Most prominent are the orange elongated rectangular crystalline or proteinaceous objects, representing degenerating ductal and/or oncocytic epithelial cell aggregates in a background of cellular debris, blood and focal regressive metaplastic changes. The cytological findings are not typical of WT. These crystalline or proteinaceous crystal-like objects appear deep blue (11,14). These structures are non-birefringent and are considered to represent condensed salivary secretory proteins, most probably α-amylase derived from salivary gland epithelial cells (14). The differential diagnosis includes oncocytoma or malignant lymphoma (11).

Basal cell adenoma accounts for 2% of all salivary gland tumors and typically presents in the 6th decade of life, an age range of 30-80 years (11). Cytologically, a cell-rich punctate pattern is observed with uniform small basaloid cells forming cohesive clusters with a palisade-like nuclear arrangement. A hyaline stroma is visible in the background. Immunohistochemistry shows positivity for pancytokeratin in all tumor cells and positivity for p63, smooth muscle actin (SMA) and calponin in the peripheral palisade-like cells (15,16). Differential diagnoses include adenoid cystic carcinoma or basal cell carcinoma, where more cell dissociation and coarser features are observed (11).

Cytologically, oncocytoma is a benign salivary gland tumor composed of oncocytes. The cells show granular eosinophilic cytoplasm in sheets, papillary structures and single cells. Cytological atypia is minimal, characterized by small, uniform nuclei with smooth contours, fine chromatin, inconspicuous nucleoli, and absence of significant pleomorphism or mitotic activity (11).

Squamous cell carcinoma is rare as a primary tumor in the salivary glands and is more likely to occur as part of the squamous differentiation of another salivary gland carcinoma or as a metastasis. Cytologically, cells with basaloid morphology or keratinized cells are found individually or in clusters. Characteristic features include polygonal and tail-like cells, also called snake cells (14). The cytoplasm is stratified and orangophilic. The background often includes detritus, necrosis and inflammation. The differential diagnosis includes necrotizing sialometaplasia (salivary gland infarction) (17).

Adenoid cystic carcinoma (ACC) is a malignant salivary gland tumor with myoepithelial and ductal differentiation, characterized by an indolent but aggressive clinical course, with local recurrence rates of 16-67% and distant metastases in 20-64% of cases, most commonly affecting the lungs (18). The growth pattern may be glandular-cribriform, tubular or solid basaloid. Cytologically, hyaline PAS-positive globules are typical, round to irregular, glassy (hyaline) eosinophilic extracellular or intracellular structures that stain positively with the PAS reaction, indicating a high content of carbohydrate-rich material. In addition, small uniform basaloid cells with minimal cell dissociation and cystic changes may be observed, typically associated with metachromatic spheres or cylinders of cellular hyaline stoma. Neither cytological features of high-grade malignancy nor keratinization are seen

(Fig. 1J and K). Immunohistochemically, the inner epithelial cells are positive for CD117, while the myoepithelial cells are positive for SMA and p63. Molecular genetic analysis reveals t(6;9) translocations and, less frequently, t(8;9) translocation and fusions of the MYB or MYBL1 oncogenes in >80% of cases (11,18). The main entities to be distinguished from adenoid cystic carcinoma are pleomorphic adenoma, polymorphic adenocarcinoma, epithelial-myoepithelial carcinoma and basal cell adenocarcinoma (19).

Mucoepidermoid carcinoma (MEC) is a malignant salivary gland tumor composed of three distinct cell types: Mucinous, intermediate and squamous. Tumor cells are classified as either low- or high-grade lesions (11). Immunohistochemically, the epidermoid cells are positive for CK5/6 and p63. Cytologically, MEC may appear cystic and acellular with mucin in the background. Clusters of pale intermediate or epithelial cells with mucus-secreting cells are typically observed (11). Molecular genetics typically show a t(11;19) translocation and a CRTC1-MAML2 gene fusion (20,21). A rare translocation t(6;22) (p21; q12) is also found. CRTC1-MAML2 gene fusion is a chimeric (fusion) gene created by a chromosomal translocation, most commonly t(11;19) (q21; p13), in which parts of two separate genes become abnormally joined (11).

Adenocarcinoma, not otherwise specified, is the third most common carcinoma of the salivary gland and lacks features that allow classification into a specific tumor entity, included in the figures is the histologic sample of this case (11).

Acinar cell carcinoma is common in the parotid gland (80% of cases). It can occur at any age, with a reported mean age of diagnosis of 50 years. Cytologically, acinar cell carcinoma presents with cell-rich preparations with a monomorphic pattern and a clear background. Acinar cell clusters with fine granules in the cytoplasm show typical PAS positivity. The nuclei do not show atypia. Immunohistochemically, it is positive for discovered on GIST-1 (DOG-1) and weakly positive for CK7 (22,23).

Secretory carcinoma (new in the 2017 World Health Organization classification) is analogous to mammary carcinoma and is considered a low-grade malignancy typically found in the parotid gland (5). Cytologically, papillary structures with epithelial cells showing vacuoles and metachromatic granules are observed. Immunohistochemically, the tumor is positive for S100 and mammaglobulin but negative for DOG-1 (11). Molecular cytogenetics shows an ETV6-NTRK3 fusion (24).

Clear cell carcinoma aspirates show groups of cohesive small and large epithelial cells with prominent cell borders and uniform, round to ovoid nuclei with granular chromatin, small nucleoli and abundant cleaved cytoplasm (14). PAS staining is positive. Clear cell carcinoma shows consistent EWRS1-ATF1 gene fusion, which is a chimeric oncogenic fusion gene formed by a chromosomal translocation, most commonly t(12;22) (q13;q12), in which parts of two genes become abnormally joined (25).

Basal cell adenocarcinoma smears are cellular and show basaloid clusters of monomorphic small cells with scant cytoplasm and frequently naked nuclei. The stroma is sparse to absent: In the membranous type, it consists mainly of hyaline matrix (11). A subset of these tumors, mainly of the membranous type, is associated with multicentricity and a higher recurrence rate (26).

Salivary duct carcinoma smears are cellular and typically contain 3D clusters, sometimes with papillary configurations and flat sheets of epithelial tumor cells with necrotic backgrounds. The tumor cells are large and polygonal with abundant cytoplasm. The nuclei are pleomorphic with prominent nucleoli (11). Salivary duct carcinoma is typically characterized by ERBB2 (HER2) amplification and androgen receptor expression rather than pleomorphic adenoma gene 1 (PLAG1) or high mobility group AT-hook 2 (HMGA2) rearrangements (11). PLAG1 (a transcription factor gene that becomes oncogenic when rearranged, leading to overexpression and promotion of tumor growth) and/or HMGA2 (a chromatin remodeling gene involved in regulation of proliferation; rearrangements lead to abnormal expression) rearrangements are identified in most cases of salivary duct carcinoma arising in pleomorphic adenoma (27).

Myoepithelial carcinoma aspirate smears show a mixture of spindled and/or plasmacytoid cells in small groups or large fragments. The nuclei may be round or oval with variable cytoplasmic features (11). The immunohistochemical expression of S-100 and SOX10 is an important diagnostic feature in myoepithelial carcinoma. EWSRI gene rearrangement has been described in myoepithelial carcinomas with aggressive features and frequent necrosis (28).

Carcinoma ex pleomorphic adenoma smears typically show features of pleomorphic adenoma or high-grade adenocarcinoma; rarely, both components are defined (11). Carcinoma ex pleomorphic adenoma often harbors PLAG1 and HMGA2 rearrangements, reflecting its origin from pleomorphic adenoma (29). Although molecular pathology is increasingly relevant in salivary gland tumors, its use remains largely confined to diagnostic support (30).

The primary aim of the present study was to evaluate the diagnostic performance of FNAC in determining the histogenesis of salivary gland lesions based on cytomorphological features in a single-center cohort. Secondary objectives included comparison of cytological findings with histopathological outcomes, identification of diagnostic pitfalls and comparison with the existing literature. By integrating these aspects, the present study aimed to refine diagnostic criteria and contribute to improved diagnostic accuracy in salivary gland cytopathology.

Materials and methods

Patients. A total of 544 patients, including 283 females (52%) and 261 males (48%), between 18 and 86 years old. FNAC specimens from salivary gland lesions and other head and neck swellings were analyzed at the Institute of Pathology, Hannover Medical School (Hannover, Germany), between January 2000 and December 2024. The criteria for inclusion in the study were as follows: i) Presence of a clinically and/or radiologically confirmed salivary gland lesion (parotid, submandibular, sublingual or minor salivary glands); ii) performance of FNAC of the lesion; and iii) availability of adequate cytological material for evaluation. The exclusion criteria were as follows: i) Inadequate or non-diagnostic aspirates (e.g., blood only, insufficient cellularity); ii) previously treated lesions (for example, after radiotherapy or chemotherapy) that could affect cytological interpretation; and iii) recurrent tumors (depending on study design, often excluded or analyzed separately).

Cytological samples were obtained using standard FNAC techniques. After clinical and radiological assessment, lesions were aspirated using a 23-25-gauge needle attached to a syringe under manual negative pressure. Multiple passes were performed when necessary to ensure adequate cellularity. Smears were prepared and routinely stained using May-Grünwald-Giemsa (MGG). In selected cases, with diagnostic uncertainty or suspicion of mucinous, glycogen-rich, or extracellular matrix-producing lesions, additional PAS staining was performed to evaluate cytoplasmic and extracellular components. When available, cell blocks were prepared from cytological material and processed according to standard histological protocols (31).

Cytological diagnoses were associated with corresponding histopathological findings where surgical specimens were available. Immunohistochemical analyses were performed on selected cases with diagnostic uncertainty, indeterminate cytomorphological features, or when further tumor classification was required, using cell block material, cytospin preparations or alcohol-fixed smears. Alcohol-fixed smears were prepared using standard laboratory conditions (ethanol fixation at room temperature for routine diagnostic processing), according to established cytology protocols (32). Staining was performed using an automated immunostaining system (BenchMark, Ventana/Roche Diagnostics) following the manufacturer's protocols.

MGG staining. Air-dried smears were fixed in MG (in methanol) for 3-5 min, then stained with 1:1 mixture of MG and buffer (pH 6.8) for 5 min and Giemsa stain (1:20-1:30) for 10-20 min. All steps were performed at room temperature (20-25˚C). Samples were rinsed with water and examined, using a bright-field light microscope (Zeiss-Microscope).

PAS smears were fixed in 95% ethanol (or 10% neutral buffered formalin) for 10-15 min at room temperature (20-25˚C), rinsed in distilled water, oxidized with 0.5-1.0% periodic acid for 5.0-10.0 min, rinsed in distilled water, stained with Schiff reagent for 10.0-20.0 min, washed in running tap water for 10.0 min, counterstained with hematoxylin for 1.0-2.0 min, then rinsed in water, dehydrated through graded alcohols, cleared in xylene, and mounted.

Immunohistochemistry. Cytological material (cell blocks, cytospin preparations, or direct smears) was fixed using either formalin- or alcohol-based fixatives at room temperature. The specimens were prepared on positively charged glass slides, with cell block sections cut at a thickness of 3-4 μm. Air-dried smears were rehydrated and post-fixed in 10% formalin for 10 min. Slides were baked at 60˚C for 30-60 min. On the BenchMark stainer the slides were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval according to the standard automated immunohistochemistry (33). Antigen retrieval was carried out using CC1 (pH 8.5) or CC2 (pH 6) for 32-64 min at 95˚C. Endogenous peroxidase was blocked using H_2O_2. The primary antibody was then applied for 16-32 min, followed by detection with the ultraView or OptiView DAB system and counterstaining with hematoxylin at room temperature. The immunohistochemical stains were performed using ready-to-use primary antibodies (Roche Tissue Diagnostics) on an automated staining platform (BenchMark ULTRA; Roche Tissue Diagnostics). The exact antibody concentrations are not disclosed by the manufacturer for these pre-diluted reagents. Staining was performed according to the manufacturer's optimized protocols.

Table I. Total number of cases with different salivary gland lesions.

Diagnosis	Number of cases	%
Benign	422	77.57
Malignant	59	10.85
Normal	26	4.78
Non-representative	37	6.80

Primary antibodies were applied at manufacturer-recommended dilutions (catalogue number and supplier specified for each antibody, Roche Tissue Diagnostics) and incubated for 30-60 min at room temperature (or overnight at 4˚C when indicated). After washing in phosphate-buffered saline, slides were incubated with appropriate secondary antibodies (enzyme-conjugated, typically horseradish peroxidase-labelled systems; catalogue number and supplier specified, Roche Tissue Diagnostics) for 20-30 min at room temperature. Immunohistochemistry was performed using a panel of primary antibodies, including: CK7 (clone OV-TL 12/30), AE1/AE3 (pan-cytokeratin cocktail AE1/AE3/PCK26), CK5/6 (clone D5/16 B4), p63 (clone 4A4), DOG1 (clone SP31), WT1 (clone 6F-H2), smooth muscle actin (SMA; clone 1A4), mammaglobin (clone 31A5), HER2/neu (clone 4B5), S-100 protein [clone (if available)], SOX10 [clone (if available)], and Ki-67 (clone MIB-1). All antibodies were applied as ready-to-use reagents on an automated Ventana BenchMark ULTRA staining platform (Roche Tissue Diagnostics) according to the manufacturer's standardized protocols. As these are proprietary, pre-optimized diagnostic reagents, exact antibody concentrations, working dilutions, and catalogue numbers are not disclosed by the manufacturer. Visualization was performed using diaminobenzidine as a chromogen. Sections were counterstained with hematoxylin for 1-2 min at room temperature, followed by dehydration through graded alcohols, clearing in xylene, and mounting. Stained slides were evaluated using a bright-field light microscope.

Results

Patients. A total of 544 salivary gland FNAC specimens (Table I) were evaluated. Patients included 283 females (52%) and 261 males (48%). FNAC demonstrated a relatively high diagnostic value in cases with representative material, particularly in distinguishing benign and malignant lesions, as evidenced by comparison with the corresponding histopathological diagnoses in cases where surgical follow-up was available. Of all cases (Table I), 422 (77.6%) were benign, 59 (10.80%) were malignant, 26 (4.80%) were normal (Fig. 1A) and 37 (6.80%) were non-representative (the sample did not adequately reflect the lesion that was targeted, so it is not suitable for diagnosis). Among 422 benign lesions (Table II), 174 (41.20%) were inflammatory lesions (Fig. 1B and C), 108

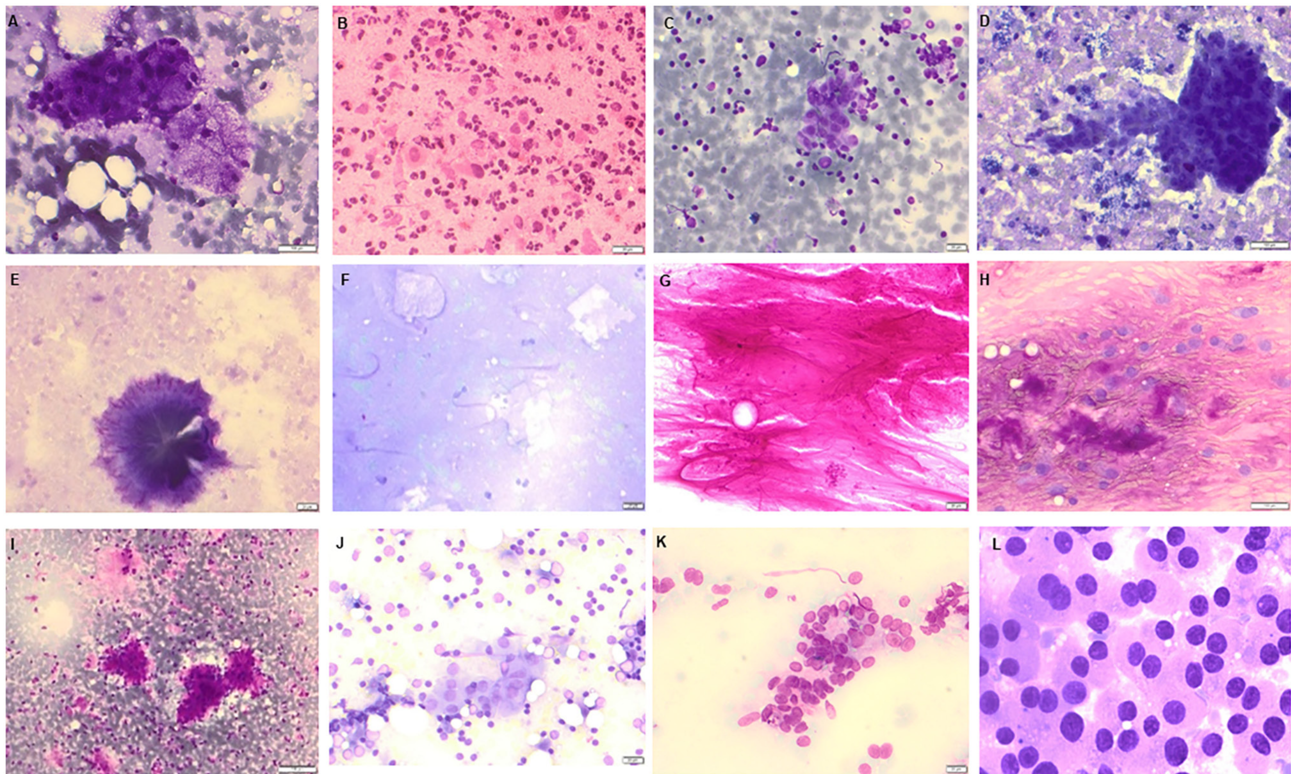

Figure 1. (A) Normal cells of the salivary gland (magnification, x25). (B) Acute sialadenitis (magnification, x25). (C) Chronic sialadenitis (magnification, x20). (D) Cyst with actinomyces (magnification, x20), (E) a cystic cavity or dilated duct, colonized by *Actinomyces* species, and often associated with chronic suppurative inflammation (magnification, x20), and (F) a cyst with actinomyces and crystal gaps (magnification, x20). (G) Previous or resolved bleeding (magnification x20). (H) Pleomorphic adenoma with myxoid background (magnification, x20). (I) Myxoid background, epithelial and myoepithelial cell groups in pleomorphic adenoma (magnification, x20). (J) Warthin's tumor, Lymphocytes and oncocytic cells (magnification, x25). (K) Basal cell adenoma (magnification, x20). (L) Oncocytic cells from oncocytoma (magnification, x25).

(25.60%) were cysts (Fig. 1D-F), 72 (17.10%) were pleomorphic adenoma (Fig. 1H and I), 64 (15.20%) were Warthin's tumor (Fig. 1J), (0.50%) were basal cell adenoma (Fig. 1K) and 2 (0.50%) were oncocytoma (Fig. 1L). Among 59 malignant lesions (Table III), 13 (18.64%) were adenocarcinoma (Fig. 2A and B), 3 (5.09%) were poorly differentiated carcinoma, 2 (3.40%) were acinar cell carcinoma (Fig. 2C and D), 1 (1.69%) was basal cell carcinoma, 1 (1.69%) was secretory carcinoma (Fig 2E and F), 13 (22.03%) were squamous cell carcinoma (Fig. 2G and H), 1 (1.69%) was adenoid cystic carcinoma (Fig. 2I and J), 1 (1.69%) was ductal carcinoma, 1 (1.69%) was mucoepidermoid carcinoma (Fig. 2K and L), 1 (1.69%) was myoepithepithelial carcinoma, 20 (33.92%) were non-Hodgkin lymphoma (NHL), 1 (1.69%) was malignant melanoma and 3 (5.09%) were renal cell carcinoma metastasis.

Diagnostic accuracy. Based on available histopathology (n=481 cases; excluding normal and non-representative aspirates), FNAC diagnostic performance for distinguishing benign vs. malignant lesions [Table IV: True positives (TP)=56, false negatives (FN)=3, false positives (FP)=2, true negatives (TN)=420, total=481] yielded sensitivity of 94.9% (sensitivity=TP/FN+TP=56/3+56=56/59), specificity of 99.5% (TN/TN+FP=420/420+2=420/422), positive predictive value of 96.6% (TP/TP+FP=56/56+2=56/58), negative predictive value of 99.3% (TN/TN+FN=420/420+3=420/423) and overall accuracy of 98.1% (TP+TN/total=56+420/481). False-negative cases (n=3) included low-grade mucoepidermoid carcinoma, small acinar cell carcinoma and poorly differentiated carcinoma where cellularity was insufficient. There were two false-positives: Reactive lymphoid hyperplasia mimicking lymphoma and severely inflamed Warthin's tumor misinterpreted as malignant.

Certain tumor entities, such as adenoid cystic carcinoma, presented greater diagnostic challenges, with lower detection rates in the range of 13 to 50%. In these cases, overlapping cytomorphological features and limited cellularity contributed to diagnostic uncertainty. Overall, FNAC findings were most reliable when correlated with histopathological results and supported by clinical and radiological information. The 5-year survival rate (Table V) varies depending on the tumor type, and late recurrence is common (35-79%).

Discussion

The present study highlighted the diagnostic utility of FNAC in the evaluation of salivary gland lesions, while also emphasizing its limitations. The present series confirmed the predominance of benign (77.6%) over malignant lesions (10.8%), consistent with previous reports (1,11,15). Inflammatory lesions and cysts were the most frequent benign lesions, while NHL represented a notable portion of malignancies. FNAC demonstrated high diagnostic accuracy (overall, 98.1%), specificity (99.5%) and sensitivity (94.9%). False-negative results occurred primarily

Table II. Benign lesions of the salivary gland (n=422).

Diagnosis	Number of cases	%
Inflammation	174	41.23
Cyst	108	25.60
Pleomophic adenoma	72	17.06
Warthin's tumor	64	15.17
Basal cell adenoma	2	0.47
Oncocytoma	2	0.47

Table III. Malignant tumors of the salivary gland (n=59).

Diagnosis	Number of cases	%
Squamous cell carcinoma	13	22.03
Adenocarcinoma	11	18.64
Poorly differentiated carcinoma	3	5.09
Acinar cell carcinoma	2	3.40
Basal cell carcinoma	1	1.69
Ductal cell carcinoma	1	1.69
Myoepithelial carcinoma	1	1.69
Mucoepidermoid carcinoma	1	1.69
Adenoid cystic carcinoma	1	1.69
Secretory carcinoma	1	1.69
Non-Hodgkin lymphoma	20	33.92
Malignant melanoma	1	1.69
Renal cell carcinoma	3	5.09

Table IV. Fine-needle aspiration cytology diagnostic performance for distinguishing benign vs. malignant lesions.

	FNAC diagnosis		
Histopathology	Malignant	Benign	Total
Malignant	56 (TP)	3 (FN)	59
Benign	2 (FP)	420 (TN)	422

TP, true positive; FP, false positive; FN, false negative; TN, true negative.

in low-cellularity tumors or cystic lesions, emphasizing the need for adequate sampling. False positives were rare but highlight potential pitfalls in inflammatory or reactive lesions.

The relatively wide range of sensitivity and specificity observed between the present and previous studies reflects the inherent complexity and morphological diversity of salivary gland pathology (11,15,34). However, FNAC accuracy is dependent on several factors, including sampling technique, preparation quality and the experience of the cytopathologist. In addition, the integration of clinical history and imaging findings serves a crucial role in accurate interpretation.

Table V. Overall, 5-year survival of patients with salivary gland tumors.

Salivary gland tumor	5-year survival rate, %
Acinar cell carcinoma	80
Mucoepidermoid carcinoma	
Low-grade	<70
High-grade	<50
Adenocarcinoma, not otherwise specified	50-70
Squamous cell carcinoma	40-55
Undifferentiated Carcinoma	30-40
Carcinoma in pleomorphic adenoma	50-70

FNAC is used as a preoperative diagnostic modality for salivary gland lesions due to its minimal invasiveness, rapid turnaround and relatively low cost. Numerous studies (11,15) have demonstrated its overall high diagnostic performance, especially for distinguishing benign from malignant processes, although sensitivity and specificity vary substantially across patient populations and institutional settings. For example, systematic reviews have shown that sensitivity in identifying malignancy ranges widely and that FNAC generally achieves high specificity in salivary gland lesions, although notable heterogeneity exists among studies (34,35).

The reported sensitivity and specificity of FNAC in salivary gland lesions shows variability, ranging from 60.0 to 97.5%, depending on lesion type and sample quality (15,34,35).

In a clinical series comparing FNAC with histopathology, sensitivity and specificity values have ranged from 50 to >90 %, depending on lesion type, sampling adequacy and interpretive criteria (15,33,34). These findings are consistent with the present study, where FNAC demonstrated a relatively high diagnostic value in representative material with variable performance metrics (including sensitivity, specificity, positive predictive value and negative predictive value). Factors contributing to this variability include the intrinsic morphological diversity of salivary gland tumors, sampling error in cystic or hypocellular lesions and interpretive challenges posed by tumors with overlapping cytomorphological features (11,15,34).

The cytological interpretation of certain entities, such as adenoid cystic carcinoma and other types of basaloid neoplasm, remains particularly challenging due to subtle morphological features that may be underrepresented in aspirate. Moreover, low-grade malignancies, cystic tumors, and heterogeneous tumors such as pleomorphic adenomas can yield hypocellular or misleading cytological specimens, further decreasing sensitivity (11,15,34). These limitations underscore the importance of associating FNAC findings with clinical presentation and imaging studies to optimize diagnostic accuracy (35).

Several authors (11,15) have also highlighted the role of adjunctive techniques such as immunocytochemistry and molecular diagnostics in improving diagnostic specificity and subclassification, particularly when cytomorphology is equivocal. However, these techniques are typically limited by sample quantity and are not necessary in straightforward

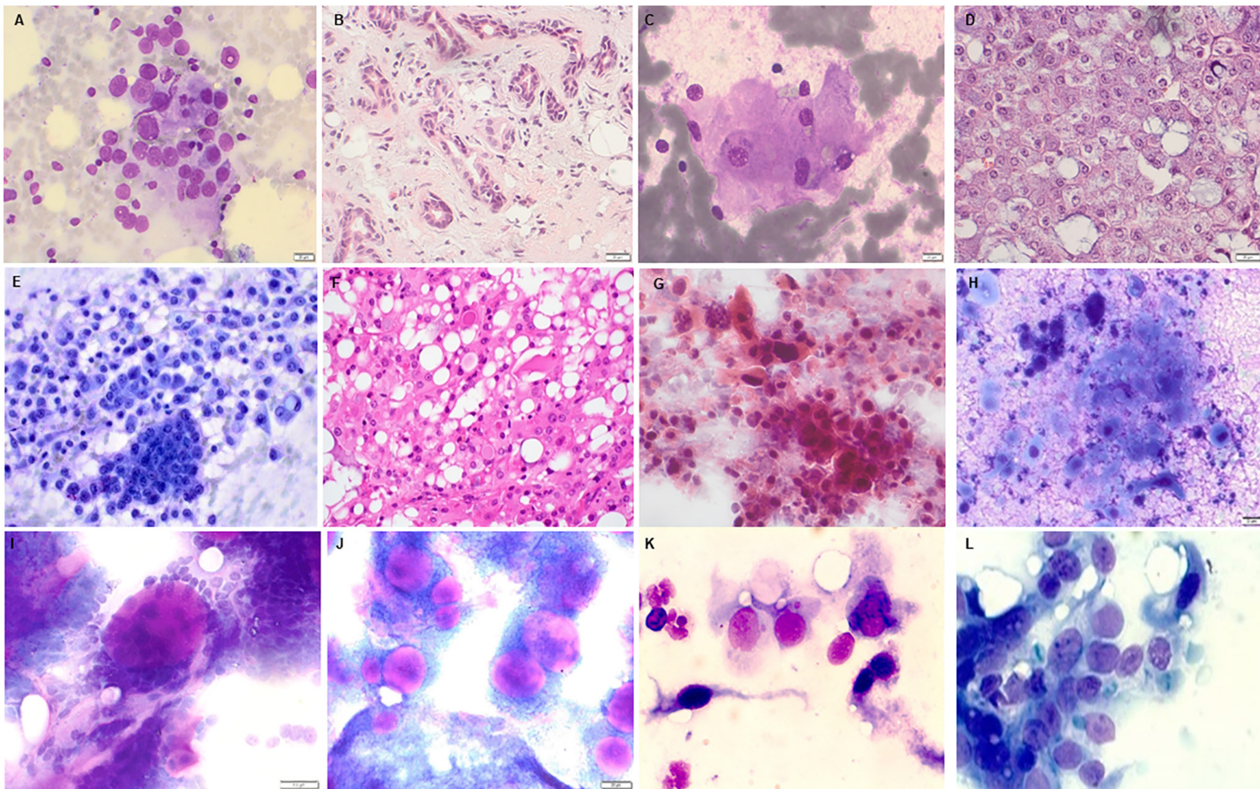

Figure 2. (A) Adenocarcinoma NOS (MGG; magnification, x25); (B) adenocarcinoma NOS of the salivary gland (H&E; magnification, x20); (C) acinus cell carcinoma (MGG; magnification, x25); (D) acinus cell carcinoma (H&E; magnification, x20); (E) secretory carcinoma (MGG; magnification, x25); (F) secretory carcinoma (H&E; magnification, x20). (G) Squamous cell carcinoma (magnification, x40); (H) inflammatory cells and necrotic cells in squamous cell carcinoma (magnification, x40); (I) adenoid cystic ca. with globules (magnification, x40); (J) adenoid cystic carcinoma (magnification, x25); (K) mucoepidermoid carcinoma (magnification, x40); (L) malignant cells of mucoepidermoid carcinoma (magnification, x40). NOS, not otherwise specified; H&E, hematoxylin and eosin; MGG, May-Grünwald-Giemsa,

cases (36). Therefore, although ancillary studies contribute valuable information, cytomorphological assessment remains the cornerstone of FNAC interpretation.

In recent years (11,30,37,38), cytology specimens have increasingly been used for molecular testing to complement conventional cytomorphological assessment. FNA material, particularly cell blocks, is used for immunohistochemistry, fluorescence *in situ* hybridization and NGS to detect gene fusions, mutations and other molecular alterations relevant for tumor classification and, in certain cases, targeted therapy. For example, the TS variant transcription factor 6-eurotrophic receptor tyrosine kinase 3 fusion (ETV6-NTRK3; ETV6-NTRK3 fusion is a gene rearrangement resulting from a translocation between chromosomes 12 and 15, leading to the formation of a constitutively active tyrosine kinase fusion protein that drives oncogenic signaling. It is the defining molecular alteration of secretory carcinoma of the salivary gland) or MYB/MYBL1 rearrangements (MYB or MYBL1 rearrangements refer to gene fusions or translocations involving the MYB or MYBL1 transcription factor genes, resulting in overexpression of oncogenic transcriptional programs. These alterations are characteristic molecular events in adenoid cystic carcinoma and are associated with tumor development and progression) are reliably identified in FNA-derived material, enabling more precise subclassification and improved diagnostic confidence (11,18,20). However, the use of cytology samples for molecular analysis has limitations. Adequate cellularity is key and cystic or low-cellularity lesions may yield insufficient DNA/RNA. Fixatives such as alcohol or formalin, commonly used for smears and cell blocks, may also affect nucleic acid quality (30,36,38). Nevertheless, when sufficient material is available, molecular testing on cytology specimens provides key diagnostic and prognostic information, especially in cases where surgical tissue is unavailable or limited. Integrating molecular testing with conventional cytology thus enhances diagnostic yield and supports personalized patient management (11,30,38).

Here, NHL accounted for approximately one-third of malignant salivary gland lesions, making it the most frequent malignancy in the present cohort. This high prevalence contrasts with published reports (39,40), where epithelial malignancies such as mucoepidermoid carcinoma, adenoid cystic carcinoma and adenocarcinoma typically predominate. The elevated proportion of NHL in the present study may reflect the referral patterns to our tertiary center (Institute for Pathology, Hannover medical School, Hannover, Germany), which frequently receives cases of lymphoproliferative disorders requiring cytological evaluation. Cytologically, NHL poses diagnostic challenges, as cellular smears can mimic reactive lymphoid hyperplasia or other small round cell tumors, increasing the risk of false-negative or ambiguous results. FNAC, combined with immunophenotyping on cell blocks (CD20, CD3, Ki-67), allows reliable subclassification and differentiation from benign lymphoid proliferations,

guiding management. Molecular investigations and clonality testing performed on the cell block support the diagnosis of malignant lymphoma. These findings underscore the importance of considering lymphoid malignancy in the differential diagnosis of salivary gland masses, particularly in older adults, where primary salivary gland lymphomas are more prevalent, and in patients with systemic (B) symptoms suggestive of hematologic disease. This highlights the role of FNAC as a first-line, minimally invasive diagnostic tool (39,40).

In summary, the literature (11,15,28) supports the use of FNAC as a valuable component of the diagnostic pathway for salivary gland lesions, particularly when integrated within a multidisciplinary framework including clinical evaluation and imaging. Such an integrated approach improves diagnostic yield and decreases limitations of cytological interpretation alone.

In the present study, FNAC, when combined with cytomorphological assessment and clinical-radiological findings, allowed reliable histogenetic interpretation in a notable proportion of cases.

FNAC is a useful first-line diagnostic tool in the evaluation of salivary gland lesions, particularly for guiding the distinction between benign and malignant entities. Its diagnostic performance is influenced by lesion type, specimen adequacy and interpretative expertise, which accounts for variability in accuracy across different diagnostic categories. For optimal diagnostic reliability, FNAC should be applied within a structured multidisciplinary framework that integrates cytological, clinical and radiological findings.

Acknowledgements

The authors would like to thank Mrs Petra Abbas (Freelancer; Greven, Germany) for proofreading.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BS contributed to the conception and design of the study. BS developed the methodological framework, including cytological, histopathological and molecular procedures. BS performed and/or reviewed the cytological and histopathological assessments. BS supervised molecular pathological analyses, including NGS and related techniques. MA contributed to the conception and design of the study. MA performed and/or reviewed the cytological and histopathological assessments. MA drafted the manuscript. BS and MA were responsible for collection and processing of clinical, cytological, and histopathological data. BS and MA analyzed and interpreted the data and contributed to the evaluation of diagnostic performance. All authors have read and approved the final manuscript. BS and MA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The study was reviewed by the Ethics Committee of Hannover Medical School (approval no. 18.02.2025; Hannover, Germany), which waived the requirement for ethical approval and written informed consent due to the retrospective and fully anonymized nature of the data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Eveson JW and Cawson RA: Salivary gland tumours. A review of 2,410 cases with particular reference to histological types, site, age and sex distribution. J Pathol 146: 51-58, 1985.
2. Aher TS, Ekhar VR, Shelkar RN and Vedi JN: Salivary gland lesions: A clinico-pathological correlation. Indian J Otolaryngol Head Neck Surg 75: 1665-1669, 2023.
3. Venkatesh S, Srinivas T and Hariprasad S: Parotid gland tumors: 2-year prospective clinicopathological study. Ann Maxillofac Surg 9: 103-109, 2019.
4. Tani EM and Skoog L: Salivary glands and rare head and neck lesions. In: Comprehensive Cytopathology. Bibbo M and Wilbur DC (eds). 3rd edition. Saunders Elsevier, Philadelphia, PA, pp607-632, 2008.
5. El-Naggar AK: WHO classification of tumors of salivary glands. In: WHO Classification of Head and Neck Tumours. El-Naggar AK, Chan JKC, Grandis JR, Takata T and Slootweg PJ (eds). 4th edition. IARC, Lyon, p160, 2017.
6. Klijanienko J, Vielh P, Batsakis JD, el-Naggar AK, Jelen M and Piekarski JD: Salivary gland tumours. Monogr Clin Cytol 15: III-XII, 2005.
7. Faur AC, Buzaș R, Lăzărescu AE and Ghenciu LA: Current developments in diagnosis of salivary gland tumors: From structure to artificial intelligence. Life (Basel) 14: 727, 2024.
8. Saoud C, Bailey GE, Graham A, Bonilla LM, Sanchez SI and Maleki Z: Pitfalls in salivary gland cytology. Acta Cytol 68: 194-205, 2024.
9. Carillo AM, DeLuca C, Pisapia P, Vigliar E, Ikenberg K, Freiberger SN, Troncone G, Rupp NJ and Bellevicine C: Molecular testing in salivary gland cytopathology: A practical overview in conjunction with the Milan system. Cytopathology 35: 330-343, 2024.
10. Kessler AT and Bhatt AA: Review of the major and minor salivary glands, Part 1: Anatomy, infectious, and inflammatory processes. J Clin Imaging Sci 8: 47, 2018.
11. El-Naggar AK, Chan JKC, Grandis JR, Takata T and Slootweg PJ (eds): WHO Classification of Head and Neck Tumours. 5th edition. IARC, Lyon, 2022.
12. Pantanowitz L, Thompson LDR and Rossi ED: Diagnostic approach to fine needle aspirations of cystic lesions of the salivary gland. Head Neck Pathol 12: 548-561, 2018.
13. Ersöz C, Cetik F, Aydin O, Cosar EF and Talas DU: Salivary duct carcinoma ex pleomorphic adenoma: Analysis of the findings in fine-needle aspiration cytology and histology. Diagn Cytopathol 19: 201-204, 1998.
14. Sáenz-Santamaría J, Catalina-Fernandéz I and Fernandez-Mera JJ: Sialadenitis with crystalloid formation. Fine needle aspiration cytodiagnosis of 15 cases. Acta Cytol 47: 1-4, 2003.
15. Faquin WC and Rossi ED (eds): The Milan System for Reporting Salivary Gland Cytopathology. Springer International Publishing, Cham, 2018.
16. Bhat A, Rao M, Geethamani V and Shetty AC: Basal cell adenoma of the parotid gland: Cytological diagnosis of an uncommon tumor. J Oral Maxillofac Pathol 19: 106, 2015.

17. Li S, Baloch ZW, Tomaszewski JE and LiVolsi VA: Worrisome histologic alterations following fine-needle aspiration of benign parotid lesions. Arch Pathol Lab Med 124: 87-91, 2000.
18. Xu B, Drill E, Ho A, Ho A, Dunn L, Prieto-Granada CN, Chan T, Ganly I, Ghossein R and Katabi N. Predictors of outcome in adenoid cystic carcinoma of salivary glands: A clinicopathologic study with correlation between MYB fusion and protein expression. Am J Surg Pathol 41: 1422-1432, 2017.
19. Mukunyadzi P: Review of fine-needle aspiration cytology of salivary gland neoplasms, with emphasis on differential diagnosis. Am J Clin Pathol 118: S100-S115, 2002.
20. Toper MH and Sarioglu S: Molecular pathology of salivary gland neoplasms: Diagnostic, prognostic, and predictive perspective. Adv Anat Pathol 28: 81-93, 2021.
21. Wakely PE Jr: Mucoepidermoid carcinoma: Analysis of 76 cytologic cases and correlation with histologic grade. Cancer Cytopathol 130: 783-799, 2022.
22. Griffith CC, Siddiqui MT and Schmitt AC: Ancillary testing strategies in salivary gland aspiration cytology: A practical pattern-based approach. Diagn Cytopathol 45: 808-819, 2017.
23. Meiklejohn K, Hrones M, Wang M, Prasad ML, Cai G, Adeniran A and Gilani SM: Diagnosis of acinic cell carcinoma of the salivary gland on cytology specimens: Role of NOR-1 (NR4A3) immunohistochemistry. Cytopathology 34: 219-224, 2023.
24. Agarwal C, Jain M, Pujani M, Rajpoot J, Goel H, Sarohi M and Sasidharan J: Secretory carcinoma of salivary gland: A rare tumor masquerading as acinic cell carcinoma on cytology. Diagn Cytopathol 52: E19-E22, 2024.
25. Hirose K, Usami Y, Kohara M, Sato S, Iwamoto Y, Murakami S, Uchihashi T, Oya K, Fukuda Y, Hori Y, *et al*: Clear cell carcinoma of palatal minor salivary gland harboring a novel EWSR1-ATF1 fusion gene: Report of a case and review of the literature. Head Neck Pathol 15: 676-681, 2021.
26. Robinson RA: Basal cell adenoma and basal cell adenocarcinoma. Surg Pathol Clin 14: 25-42, 2021.
27. Wakely PE: Salivary duct carcinoma: A report of 70 FNA cases and review of the literature. Cancer Cytopathol 130: 595-608, 2022.
28. Argyris PP and Wakely PE Jr: Cytopathology of salivary gland myoepithelial carcinoma: A study of 13 cases and review of the literature. J Am Soc Cytopathol 12: 461-468, 2023.
29. Bahrami A and Perez-Ordoñez B: Carcinoma ex pleomorphic adenoma: Pathogenesis and molecular genetics. Head Neck Pathol 8: 30-36, 2014.
30. Pusztaszeri M, Rossi ED and Faquin WC: Is molecular testing of salivary gland FNA specimens ready for prime time? Cancer Cytopathol 132: 393-395, 2024.
31. Kiernan JA: Histological and histochemical methods: Theory and practice. 5th edition. Scion Publishing, Banbury (UK), 2015.
32. Koss LG and Melamed MR: Koss' diagnostic cytology and its histopathologic bases. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
33. Dabbs DJ: Diagnostic immunohistochemistry: Theranostic and genomic applications. 6th ed. Philadelphia: Elsevier; 2022.
34. Schmidt RL, Hall BJ, Wilson AR and Layfield LJ: A systematic review and meta-analysis of the diagnostic accuracy of fine-needle aspiration cytology for parotid gland lesions. Am J Clin Pathol 136: 45-59, 2011.
35. Fouladpour N, Ali SZ and Olson MT: Accuracy of fine-needle aspiration cytology in salivary gland lesions: A systematic review and meta-analysis. Cancer Cytopathol 124: 695-709, 2016.
36. Cibas ES and Ducatman BS: Cytology: Diagnostic principles and clinical correlates. 5th ed. Elsevier; 2020.
37. -Gargano SM, Senarathne W, Feldman R, Florento E, Stafford P, Swensen J, Vranic S and Gatalica Z: Novel therapeutic targets in salivary duct carcinoma uncovered by comprehensive molecular profiling. Cancer Med 8: 7322-7329, 2019.
38. Rossi ED and Faquin WC: Molecular testing in cytopathology: Current applications and future perspectives. Cancer Cytopathol 131: 289-303, 2023.
39. Van Wal JE, Wolvius EB, Huijgens PC and van der Waal I: Malignant lymphomas of the salivary glands: A clinicopathological study. Oral Oncol 38: 403-409, 2002
40. Ellis GL and Auclair PL: Tumors of the salivary glands. AFIP Atlas of Tumor Pathology, Series 4, Fascicle 9. Washington, DC: American Registry of Pathology; 2008.

Copyright © 2026 Soudah and Abbas. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.