MINI REVIEW

Classification of salivary gland lesions on cell block preparations with a panel of immunohistochemical markers - a rapid, reliable, and minimally invasive diagnostic modality

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ABSTRACT

Fine-needle aspiration cytology (FNAC) is used as a valuable method for examining suspected salivary gland lesions. It is a simple, cost-effective, and minimally invasive procedure with high specificity and sensitivity. Due to the cellular heterogeneity and overlapping architectural features, it can be difficult to distinguish between non-neoplastic processes, benign lesions, and/or malignancies in salivary glands on routine stains. Cell block methods are currently replacing surgical biopsy-based diagnostic methods on the basis of utilizing aspirates from FNAC with a rapid and reliable potential for reaching a conclusive diagnosis. Ancillary investigations including a panel of immunohistochemical markers are frequently applied on cytology specimens in the era of precision diagnostics to offer a specific diagnosis and even prognostic information for optimal patient care.

Keywords: Cell block, salivary glands, Milan system, cytopathology, immunohistochemistry.

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Salivary gland tumors (SGTs) are a variety of neoplasms in the head and neck region with diverse histological features and clinical manifestations. In the maxillofacial region, these tumors are quite uncommon, accounting for 3%-10% of all neoplasms and roughly 6% of head and neck cancers.[1] Despite large differences in the incidence recorded across the world, these carcinomas have a reported incidence of around 1.1 cases per 100,000 people in the United States[2] while most benign SGTs have an average incidence of 0.4-13.5 cases per 100,000 population.[3] The epidemiological analysis of SGTs by The Armed Forces Institute of Pathology in Rawalpindi, Pakistan reports pleomorphic adenoma (PA) and adenoid cystic carcinoma (AdCC) as the most frequent benign and malignant SGTs, respectively.[1] Although the tumors of the salivary gland can appear at any age; however, a predilection for the fifth to seventh decades and female gender is seen.[4]

Radiographic imaging and clinical examination alone are unable to differentiate between benign and malignant SGTs.[5] Therefore, fine-needle aspiration cytology (FNAC) is used as a valuable method for examining suspected salivary gland lesions. It is a simple, cost-effective, and minimally invasive procedure with a high specificity and sensitivity.[6]

To assess salivary gland lesions, fine-needle aspiration (FNA) is a widely used method.[7,8] It is reported to be 79% sensitive and 96% specific for diagnosing malignancy and up to 96% sensitive and 98% specific for detecting benign neoplasia of salivary glands.[9] Due to the cellular heterogeneity and overlapping architectural features, it can be difficult to distinguish between non-neoplastic processes, benign lesions, and/or malignancies on routine stains, even though the majority of frequently occurring salivary gland neoplasms [such as pleo PA or Warthin tumor (WT)] pose little diagnostic challenges on FNA.[10] Ancillary investigations are frequently carried out on cytology specimens in the era of precision diagnostics to offer a specific diagnosis and even prognostic information for optimal patient care.[11]

The Milan system for reporting salivary gland cytopathology (MSRSGC), introduced in 2018, established six distinct

Table 1. *Milan system (2018) for reporting salivary gland cytopathology.*

Category	Definition
Nondiagnostic	Insufficient material (either quality or quantity) to make a diagnosis. This category includes aspirates with benign elements only and nonmucinous cyst contents.
Nonneoplastic	There is no evidence of a neoplastic process. This category includes acute, chronic, and granulomatous sialadenitis as well as reactive-appearing lymph nodes.
AUS	The pathologist cannot entirely exclude a neoplasm. This category includes reactive atypia, a poorly sampled neoplasm, mutinous cyst contents, and any sample you would send for flow cytometry.
Neoplastic (benign)	Aspirate material is diagnostic of a benign neoplasm. Entities may include lipomas, WTs, PAs, and others.
SUMP	The aspirate is diagnostic of a neoplasm but not specific and a malignant neoplasm cannot be excluded. Examples include a PA with metaplasia or atypia, myoepitheliomas, and basal cell neoplasms.
Suspicious	The aspirate is highly suggestive of malignancy but not definitive. Cases in this category are usually high-grade carcinomas with limited sampling.
Malignant	The aspirate is diagnostic of malignancy.

Table 2. *The American Society of Cytopathology criteria for assessment of ROM and therapeutic approach for MSRSGC.*

Diagnostic category	Risk of malignancy (%)	Management
I. Non-diagnostic	25	Clinical and radiologic correlation/repeat FNAC
II. Non-neoplastic	10	Clinical follow-up and radiological correlation
III. Atypia of undetermined significance (AUS)	20	Repeat FNAC or surgery
IV. Neoplasm		
a) Neoplasm: Benign	<5	Surgery or clinical follow-up
b) Neoplasm: Salivary gland neoplasm of uncertain malignant potential (SUMP)	35	Surgery
V. Suspicious for malignancy (SM)	60	Surgery
VI. Malignant	90	Surgery

diagnostic categories with the associated risk of malignancy (ROM) based on cyto-morphologic features in an effort to standardize SG FNAC reporting and streamline downstream clinical management.[12] The seven diagnostic categories that make up this tier system each has a unique ROM.[13,14] With a realistic, evidence-based, user-friendly categorization system, and characterization and management algorithms, it aims to improve patient care.[15,16] The following table explains the categories of MSRSGC.[17]

The FNAC findings are classified into many diagnostic categories in the MSRSGC. In addition, the American Society of Cytopathology calculated the ROM for each group and suggested a therapeutic approach (Table 2).[15,18]

However, a significant fraction of salivary gland aspirates may provide nondiagnostic results.[19] A meta-analysis reported 10% nondiagnostic samples with 25% ROM in such aspirates although the actual ROMs reported in recent studies tend to be lower, with a mean ROM of 17% (range, 0%-50%). Several international studies have stated that the MSRSGC is a reliable tool for cytopathological categorization of salivary gland lesions.[20,21] Due to the morphological overlap between salivary gland lesions, and the relatively common (partial) cystic lesions, it is well recognized that salivary gland cytology interpretation is challenging.[8,22]

Cell blocks (CBs) are preparations in which cytologic material is collected and processed as a paraffin-embedded block in a manner that is comparable to formalin-fixed paraffin-embedded tissue in surgical pathology. Any sort of cytology sample, including leftovers from liquid-based preparations or scrapes of traditional smears, can be used to create CBs.[23] The preparation of CBs may be done in laboratories using a variety of techniques, including the HistoGel method, the Shandon Cytoblock method, the plasma thrombin CB preparation method, the Cellient automated CB system, the tissue coagulum clot method, and the formalin or alcohol vapor method. However, since the HistoGel method on CB, preparation offers a higher quality of morphological features and retained tissue architecture. The cell pellet is combined using HistoGel, a modified agar, after centrifugation of the cell suspensions. In this procedure, the HistoGel must be heated to a liquid condition, then combined with the pellet, cooled to solidify, and then immersed in formalin for histological processing. The amount of gel that is required must be carefully calculated since too little hinders cohesion and too

much dilutes the material. Now this pellet is processed to form paraffin blocks, and stained with hematoxylin-eosin like other excisional surgical specimens.[24]

CB can provide several serial tissue sections that may be utilized for ancillary studies such as immunohistochemistry, special stains, and molecular assays. These preparations can also be saved and kept for a longer time with the possibility of being used in the future for additional research or comparison with fresh diagnostic investigations.[23] Even with scant evidence, a panel of immunostains may produce a conclusive diagnosis.[25] For example, the p63 immunomarker can reliably differentiate polymorphous adenocarcinoma, a well-known tumor lacking myoepithelial cells (p63), from tumors having myoepithelial cells (p63+), such as PA and AdCC.[26]

The secretory carcinoma of SG is frequently misinterpreted as acinic cell carcinoma, PA, WT, low-grade mucoepidermoid carcinoma (MEC), adenocarcinoma not otherwise defined, and neoplasia with myoepithelial differentiation.[27] Though it comprises less than 0.3% of all SGTs, a variety of histological traits and clinical characteristics are seen in secretory carcinoma. The histopathological diagnosis of this carcinoma can be difficult with current available immunohistochemical markers.[28] Mammaglobin among these antigens has a diagnostic sensitivity of up to 95%.[29] Mammaglobin and S-100 can also be expressed positively in certain low-grade epithelial-derived tumors, such as polymorphous low-grade adenocarcinoma and low-grade salivary duct carcinoma.[30] One of the most common immunohistochemical markers that characterizes myoepithelial cells is smooth muscle actin. This marker is also used to detect myofibroblasts, which are specialized actin-containing fibroblasts that are involved in the progression of various malignant neoplasms.[31]

DOG 1, a transmembrane protein, was originally discovered in gastrointestinal stromal tumors and functions as a calcium-activated chloride channel protein. Expression of DOG-1 in salivary AciCC is reported to be 55% in a meta-analysis.[32] Ki-67 is a proliferation marker that is being used to determine proliferative activity in any malignant neoplasm the documented percentage positivity of Ki-67 in benign SG neoplasms is 5% or less and in malignant ones, it makes more than 23%-50% with a few exceptions.[33] The Ki-67 labeling index can be used as a reliable adjuvant diagnostic tool to differentiate between the subtypes and grading of certain malignant tumors, such as MEC, AdCC and AciCC, which are usually difficult to diagnose on histopathological criteria alone.[34]

The gold standard for identifying salivary gland lesions will always be histopathology.[35] However, CBs can be beneficial in the future as compared to conventional cytology for better architectural preservation of cytological material for salivary gland neoplasms.[21]

The application of MSRSGC, CBs with support from immunohistochemical markers has a high diagnostic yield in the categorization of SG lesions.

The use of these techniques in conventional laboratory settings may not only help the pathologists in reaching a conclusive diagnosis in a timely manner but will also support the patient by providing an early and accurate diagnosis of suspicious lesions and differentiating histological mimickers of malignancy from actual malignant neoplasm. Moreover, the use of a panel of immunomarkers will help in improving diagnostic accuracy which subsequently will help the clinicians in mapping a better management plan and improved survival for these patients.

Limitations of the Review Article

This review has several limitations. First, it is a mini-review with a narrative pattern, hence systematic gathering of data from literature was not utilized. Second, comparisons between biopsy and histopathology with the CB methods could not be made. Another limitation of this review is that the studies showing the sensitivity and specificity of a panel of routine immunohistochemical markers could have been added.

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List of Abbreviations

AdCC	Adenoid cystic carcinoma
CB	Cell block
FNAC	Fine-needle aspiration cytology
MSRSGC	Milan system for reporting salivary gland cytopathology
MEC	Mucoepidermoid carcinoma
PA	Pleomorphic adenoma
ROM	Risk of malignancy
SGTs	Salivary gland tumors
WT	Warthin tumor

Conflict of interest

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Authors' contributions

AN, FR: Conception and design of study, acquisition of data, drafting of the manuscript.
SA: Critical intellectual input.
RS: Acquisition of data.
ALL AUTHORS: Approval of the final version of the manuscript to be published.

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