Ancillary studies on cell blocks from fine needle aspiration specimens of salivary gland lesions: A multi-institutional study

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Abstract

Background: Ancillary studies are commonly performed on cell blocks prepared from fine-needle aspiration (FNA) specimens. There are limited studies in application of ancillary studies on cell blocks from salivary gland (SG) FNAs. This multi-institutional study evaluates the role of ancillary studies performed on cell blocks in the diagnosis of SG lesions, and their impact on clinical management.

Method: The electronic pathology archives of three large academic institutions were searched for SG FNAs with ancillary studies performed on cell blocks. The patient demographics, FNA site, cytologic diagnosis, ancillary studies, and surgical follow-up were recorded. If needed, the cytologic diagnoses were reclassified as per the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

Results: 117 SG FNA cases were identified including 3, 10, 11, 6, 23, 4, and 60 cases in MSRSGC categories I, II, III, IVa, IVb, V, VI, respectively with surgical follow-up available ranging from 27% to 100% within each category. Ancillary studies including histochemistry, immunocytochemistry (IHC), and in situ hybridization (ISH) were beneficial in 60–100% of cases in each category. Risk of malignancy was 100% in both the suspicious for malignancy (V) and malignant (VI) categories. Ancillary studies improved diagnosis in 60% of non-neoplastic cases (II, 6/10), 100% of benign neoplasm cases (IVa, 6/6), and 98.3% of malignant cases (VI, 59/60).
1 | INTRODUCTION

Fine-needle aspiration (FNA) is a well-accepted procedure to evaluate salivary gland lesions.\(^1\,^2\,^3\,^4\) It is up to 79\% sensitive and 96\% specific in detecting malignancy, and up to 96\% sensitive and 98\% specific in the detecting neoplasia, respectively.\(^5\) Although most commonly occurring salivary gland neoplasms pose little diagnostic challenge on FNA (i.e., pleomorphic adenoma or Warthin tumor), differentiating between non-neoplastic processes, benign lesions, and/or malignancies is not always achievable on routine stains due to cellular heterogeneity and overlapping architectural features.\(^6\,^7\)

In an effort to standardize SG FNA reporting and streamline downstream clinical management, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) established six distinct diagnostic categories with associated risk of malignancy (ROM) based on cytomorphologic features.\(^8\,^9\)

In the era of precision diagnostics, ancillary studies are often being performed on cytology specimens to provide a specific diagnosis and even prognostic information for optimal patient management.\(^9\)

A wide array of ancillary studies such as immunocytochemistry, fluorescence in situ hybridization (FISH), DNA or mRNA in situ hybridization (ISH) can be performed on cell blocks. Salivary gland neoplasia arises from a variety of cell types, which can be delineated utilizing immunocytochemistry. A small panel of immunostains may yield a definitive diagnosis, even with minimal material. For example, p16 a surrogate marker in diagnosing HPV-related squamous-cell carcinoma, allows for a more definitive diagnosis than cytological examination alone.\(^10\) However, cell blocks are not routinely prepared for all SG FNA cases due to utilization of aspirated material for direct microscopic examination and when cell blocks are available, they may be insufficient for ancillary studies.

In this multi-institutional retrospective study, we evaluated the utility of cell blocks with subsequent performance of ancillary studies in the diagnosis of salivary gland lesions classified according to the MSRSGC.

2 | MATERIALS AND METHODS

The study was conducted after obtaining institutional research approval in each institution. The electronic pathology archives of Massachusetts General Hospital (MGH), The Johns Hopkins hospital (JHH) (1999–2019), and Hospital of the University of Pennsylvania (HUP) (2015–2020) were retrospectively searched for FNAs of salivary glands with any ancillary studies performed on cell blocks. The inclusion criteria for case selection were all available salivary gland FNAs, in which a cell block was prepared and ancillary studies were performed. All cases had cell block slide(s) stained with the hematoxylin and eosin and additional ancillary studies. The cytology samples in this study were processed as Diff-Quik stained on air-dried slides, Papanicolaou stained alcohol-fixative slides, or Thin-Prep preparation of alcohol fixed aspirations.

Each institution reviewed its own cases individually and classified the cases into the MSRSGC categories. The ancillary studies included in this study were immunohistochemical stains, histochemical stains for detection of mucin, bacterial, fungal and mycobacterial micro-organisms and in situ hybridization. The following data points were recorded for each patient: tumor type, sex, age, biopsy site, FNA diagnosis, cytologic category per MSRSGC, type and results of ancillary studies performed, and surgical follow-up diagnosis when available. The study included the pathology report review only.

3 | RESULTS

One hundred and seventeen SG FNA specimens met the inclusion criteria. These included 67 male patients and 50 female patients, ranging in age from 2 to 92 years with a mean of 61.1 years and median of 63 years. The parotid gland was the most common site (101 lesions), followed by minor salivary glands (9 lesions), and submandibular gland (7 lesions). The MSRSGC diagnostic category distribution was as follows: 3 (2.6\%) cases as non-diagnostic, 10 (8.5\%) as non-neoplastic, 11 (9.4\%) as atypia of undetermined significance (AUS), 6 (5.1\%) as benign neoplasm, 23 (19.7\%) as salivary gland neoplasm of uncertain malignant potential (SUMP), 4 (3.4\%) as suspicious for malignancy, and 60 (51.3\%) as malignant (Figures 1A–3B).

Tables 1–7 summarize cases according to MSRSGC category, cytology diagnosis before and after the ancillary study results, ancillary studies performed on the cell block and their results including the reason for performing ancillary studies, and surgical pathology diagnosis if available.

Surgical follow-up was available in 59 cases (50.4\%), ranging from 27\% to 100\% of cases within each MSRSGC category. Ancillary studies were helpful in 60–100\% of cases in each MSRSGC category. Two out of three (66.6\%) cases in category I had surgical follow up and both were diagnosed malignant (cystic mucoepidermoid

| Conclusion: | Judicious and case-based ancillary studies performed on SG FNA cell blocks with sufficient material can improve the diagnostic yield by further characterization of the atypical/neoplastic cells, particularly in MSRSGC categories IVa-VI. |
| Keywords: | ancillary studies, cell block, fine-needle aspiration, histochemistry stains, immunohistochemistry, in situ hybridization, Milan System for Reporting Cytology, salivary gland |
carcinoma and chronic lymphocytic leukemia (CLL). Three out of ten cases (30%) in category II had surgical follow up, one was diagnosed as benign neoplasm (Rosai-Dorfman disease) and two inflammatory/reactive (benign lymph node with follicular hyperplasia and necrotizing granulomatous inflammation). In category III, 3 out of 10 cases had surgical follow up. One case was diagnosed malignant (secretory carcinoma) and two were benign neoplasm (oncocytic cystadenoma and oncocytoma). In category IVa, 3 out of 6 cases had surgical follow up and they were diagnosed benign neoplasm (two pleomorphic adenomas and one schwannoma). In category IVb, 16 out of 23 cases had surgical follow up including 7 malignant cases (one of each high-grade adenocarcinoma, mucoepidermoid carcinoma, myofibroblast sarcoma, metastatic renal cell carcinoma, secretory carcinoma, esthesioneuroblastoma, metastatic carcinoma with neuroendocrine differentiation) and 9 benign cases (4 pleomorphic adenomas, 2 myoepitheliomas, one granular cell tumor, one basal cell adenoma, and one Warthin tumor). All four category V cases were malignant on surgical follow-up (two salivary duct carcinomas, one secretory carcinoma, and one MALT [Mucosa-associated lymphoid tissue] lymphoma). In category VI, 28 out of 60 cases were confirmed malignant.

**Figure 1** Secretory carcinoma. (A) A large fragment of cohesive cells is seen. The cells are characterized by large cytoplasmic vacuoles and round, uniform nuclei (×200, Diff-Quik stain). (B) The cell block consists of large fragments of neoplastic cells containing abundant clear to eosinophilic cytoplasm (×200, H&E). (C) The tumor cells were positive for mammaglobin immunostain on cell block confirming the diagnosis (×200, Immunostain) [Color figure can be viewed at wileyonlinelibrary.com]

**Figure 2** Salivary duct carcinoma. (A) Malignant epithelial cells are seen arranged in clusters and single cells. The nuclei exhibit anisonucleosis, thick nuclear membrane, coarse chromatin, and prominent nucleoli (×400, Papanicolaou stain). (B) A cell block showed infiltrating carcinoma, which could be primary or secondary based on morphology alone (×200, H&E). (C) The tumor cells expressed strong nuclear staining for androgen receptor immunostain on the cell block confirming salivary duct carcinoma (×200, immunostain) [Color figure can be viewed at wileyonlinelibrary.com]

**Figure 3** Malignant melanoma. (A) Numerous single cells are seen on a smear. The cells contain round to oval nuclei with moderate amount of cytoplasm. Occasionally cells contain melanin pigment (×200, Diff-Quik). (B) The malignant cells were positive for Melan A on a cell block, confirming the diagnosis (×200, immunostain) [Color figure can be viewed at wileyonlinelibrary.com]
TABLE 1  MSRSGC category I: non-diagnostic, cytology diagnosis, ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable)

| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|---------------------------------|----------------------------------------|-------------------------------|-------------------------------|
| I      | Cyst contents with epithelioid cells    | Positive for HAM56              | Cyst contents with histiocytes          | No surgical follow-up         | To rule out epithelial cells and identify macrophages |
| I      | Cyst debris, mixed inflammation and epithelioid cells | Positive for CD68; Negative for AE1/AE3 | Cyst debris, mixed inflammation with histiocytes | Cystic mucoepidermoid carcinoma | To rule out epithelial cells and identify macrophages |
| I      | Salivary gland tissue with mixed inflammation | AE1/AE3 highlights normal salivary gland tissue; CD68 highlights histiocytes, and negative for S100, PAS; Ziehl Neelsen stain; Gram stain; and mucicarmine | Salivary gland tissue with mixed inflammation, no fungi, mycobacteria or bacteria identified | CLL | An infectious process is excluded |

Abbreviation: CLL; chronic lymphocytic leukemia.

TABLE 2  MSRSGC category II: non-neoplastic, cytology diagnosis, ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable)

| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|---------------------------------|----------------------------------------|-------------------------------|-------------------------------|
| II     | Caseating granulomas                   | Positive Ziehl Neelsen stain    | Caseating granulomas, mycobacterial organisms identified | No surgical follow-up         | Confirming mycobacteria organisms |
| II     | Dense fibrosis with clusters of pigmented macrophages and scant benign salivary gland tissue | Negative for Iron stain         | Dense fibrosis with clusters of pigmented macrophages (negative for Iron stain) and scant benign salivary gland tissue | No surgical follow-up         | Ruling out hemosiderin pigment |
| II     | Chronic sialadenitis                   | Negative for IgG4               | Chronic sialadenitis, negative for IgG4 | No surgical follow-up         | Ruling out IgG4 related disease |
| II     | Polymorphous lymphoid tissue with atypical lymphocytes, cannot exclude a lymphoproliferative disorder | Mix of CD3 positive T-cells and CD20 positive B-cells, AE1/AE3 highlights epidermis | Polymorphous lymphoid tissue | Benign lymph node with follicular hyperplasia | Ruling out lymphoma |
| II     | Chronic inflammation and plasmacytosis, cannot rule out a plasma cell proliferative disorder | CD3 and CD20 highlight mixed population of T- and B-Cells, respectively. C138 shows prominent plasma cell population that are polytypic by kappa and lambda. IgM shows scattered positivity. Positive for IGG4 | IgG4-related Chronic inflammation with increased plasma cells with no light chain restrictions | No surgical follow-up         | Ruling out a plasma cell proliferation disorder |
| II     | Chronic sialadenitis                   | Positive for IgG4               | Chronic sialadenitis Suggestive of IGG4-related disease | No surgical follow-up         | Confirming an IgG related process |

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TABLE 2  (Continued)

| MSRS GC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|---------|----------------------------------------|---------------------------------|--------------------------------------|------------------------------|-----------------------------|
| II      | Chronic inflammation and macrophages in a background of acellular matrix, (mucin vs. colloid) | Positive for Thyroglobulin | Chronic inflammation and macrophages in a background of colloid | No surgical follow-up | Identifying colloid |
| II      | Mostly macrophages and epithelioid cells, rare atypical cells in a background of lymphocytes, crystals and cell debris | CD68 highlights macrophages; AE1/AE3 stains rare degenerated epithelial cells; Mucicarmine is negative | Mostly macrophages in a background of lymphocytes, crystals and cell debris, compatible with the clinical and radiologic impression of cystic hygroma | No surgical follow-up | To evaluate nature of the epithelioid cells, epithelial cells versus macrophages |
| II      | Granulomatous inflammation | GMS and Ziehl Neelsen stains are negative | Granulomatous inflammation | Necrotizing granulomatous inflammation with organisms on FITE stain, consistent with atypical mycobacterial infection | Non-contributory |
| II      | Polymorphous lymphocytes with atypical lymphocytes and histiocytes, an infectious process cannot be entirely excluded | Mixed population of CD3 positive T cells and CD20 positive B cells, Warthin-Starry, Brown Hopp, and GMS special stains and AFB and spirochete immunostains are negative for bacterial and fungal organisms | Reactive lymph node, no micro-organisms identified | Rosai-Dorfman disease | To rule out an infectious process |

TABLE 3  MSRS GC category III: Atypia of Undetermined significance (AUS), cytology diagnosis, type of ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable)

| MSRS GC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|---------|----------------------------------------|---------------------------------|--------------------------------------|------------------------------|-----------------------------|
| III     | Acellular matrix (mucin vs. colloid), chronic inflammation, macrophages | Matrix positive for thyroglobulin | Colloid, chronic inflammation, macrophages | No surgical follow-up | Identifying nature of acellular material |
| III     | Atypical mononuclear cells suspicious for Hodgkin's disease | Negative for CD15 and CD30; Equivocal for CD68 and HAM56 | Atypical mononuclear cells in background of lymphocytes | No surgical follow-up | Rule out Hodgkin's disease |
| III     | Rare atypical epithelial cell, chronic inflammation, a low grade mucoepidermoid carcinoma cannot be entirely excluded | Negative for mucicarmine | Rare atypical epithelial cells, chronic inflammation | No surgical follow-up | Rule out mucoepidermoid carcinoma |
| III     | Salivary gland lesion composed of atypical epithelial cells and necrosis | Squamous cells are positive for p63 and negative for mucicarmine | Salivary gland lesion composed of atypical squamous cells and necrosis | No surgical follow-up | Identifying nature of the epithelial cells, squamous versus glandular |

(Continues)
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|--------------------------------|------------------------------------|----------------------------|-----------------------------|
| III    | Rare atypical poorly preserved epithelial cells in a background of cystic changes, cellular debris, necrosis, acute inflammation, and benign acinar tissue | Squamous cells are positive for CK5/6 | Rare atypical squamous cells in a background of cystic changes, cellular debris, necrosis, acute inflammation, and benign acinar tissue | No surgical follow-up | Identifying nature of the epithelial cells |
| III    | Atypical lymphoid cells favor reactive lymph node | CD3 and CD20 stain mixture of T-cell and B-cells respectively. BCL6 highlights scattered germinal centers, which are negative for BCL2. CD23 highlights follicular dendritic networks and mantle zone cells | Atypical lymphoid cells cannot exclude lymphoma | No surgical follow-up | To differentiate reactive lymph node versus atypical lymphoid proliferation |
| III    | Rare cells with oncocytic features admixed with inflammation, acinar cells and crystals | AE1/AE3 highlights salivary gland epithelium; mucicarmine is negative | Rare oncocytic cells with mixed inflammation, acinar cells and crystals | Oncocytic cystadenomas | To rule out intracellular mucin |
| III    | Atypical epithelial cells with focal squamous and glandular features and focal inflammation | Squamous cells are positive for CK5/6 and p63; macrophages are negative for mucicarmine | Atypical metaplastic squamous cells, favor reactive, foamy macrophages, and focal inflammation, favored a dilated salivary duct which has undergone squamous metaplasia with reactive atypia (patient has a history of treated abscess). A low grade salivary gland neoplasm with squamous metaplasia cannot be entirely excluded. | No surgical follow-up | To confirm the nature of squamous cells and evaluate mucin in vacuolated macrophages |
| III    | Atypical lymphoid cells in a background of normal salivary gland parenchyma, epithelioid cells, polarizable crystalline material and amorphous debris | CD68 highlights macrophages; negative for AE1/AE3 and S100; mucicarmine is non-contributory | Atypical lymphoid cells in a background of normal salivary gland parenchyma, histiocytes, polarizable crystalline material and amorphous debris | No surgical follow-up | To evaluate the nature of epithelioid cells |
| III    | Acellular eosinophilic material of uncertain origin (keratin vs. amyloid), squamous cells, chronic inflammation | Squamous cells and background keratin are positive for AE1/AE3; Congo red is negative for amyloid | Abundant eosinophilic necrotic and mummified material consistent with keratin and necrotic keratinized cells, few viable squamous cells without cytologic atypia, and macrophages present | Oncocytoma | Ruling out amyloid |
on surgical follow up (7 salivary duct carcinomas, 7 squamous-cell carcinomas including one HPV-related case, 5 metastatic melanomas, 2 acinic cell carcinomas, 2 mucoepidermoid carcinomas, 2 lymphoma cases; one Hodgkin disease and one follicular lymphoma, and one of each recurrent oligodendroglioma, high grade adenocarcinoma, and Merkel cell carcinoma). ROM was 100% in both the suspicious for malignancy (V), and malignant (VI) categories. A non-neoplastic (II) case representing reactive lymph node on FNA with clusters of

| TABLE 3 (Continued) |
|---------------------|
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
| III | Abundant oncocytic cells with associated blood vessels, the differential diagnosis includes a reactive lesion versus a salivary gland neoplasm versus melanoma | Positive for AE1/AE3 and CAM5.2; focally positive for SOX10, S100 and mucin stain; negative for HMB45; non-contributory for Melan A | Oncocytic cells with blood vessels, the differential diagnosis includes mucoepidermoid carcinoma versus a reactive lesion secondary to obstruction; there are no overt features of malignancy and no evidence of melanoma | Secretory carcinoma | To identify nature of the cells, epithelial and ruling out malignant melanoma |

| TABLE 4 MSRSGC category IVa: Benign neoplasm, cytology diagnosis, type of ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable) |
|---------------------|
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
| IVa | Spindle cell neoplasm favor schwannoma | Positive for S100 | Schwannoma | Schwannoma | Confirm Schwannoma |
| IVa | Oncocytic cells and lymphocytes, favor Warthin tumor, however metastatic lung adenocarcinoma cannot be entirely excluded | Negative for TTF-1 and Napsin A | Warthin tumor (History of lung adenocarcinoma noted) | No surgical follow-up | Rule out metastatic lung adenocarcinoma |
| IVa | Spindle cell neoplasm, favor schwannoma | Positive for S100; and negative for CD68 | Spindle cell neoplasm, consistent with schwannoma | No surgical follow-up | Confirm Schwannoma |
| IVa | Salivary gland neoplasm of uncertain malignant potential (SUMP), favor pleomorphic adenoma, however adenoid cystic carcinoma cannot be entirely excluded | Positive for p63; negative for CD117 | Myoepithelial rich pleomorphic adenoma | Pleomorphic adenoma | To rule out adenoid cystic carcinoma |
| IVa | Salivary gland neoplasm, favor pleomorphic adenoma | Positive for CK7: CK5/6; p63 (focal); Calponin is non-contributory | Pleomorphic adenoma | Pleomorphic adenoma | Detecting myoepithelial cells |
| IVa | Salivary gland neoplasm of uncertain malignant potential (SUMP) with oncocytic features and rare lymphocytes, favor Warthin tumor, however a malignant neoplasm such as acinic cell carcinoma cannot be entirely excluded | Oncocytes are negative for PAX-8 and DOG-1; p63 highlights basal cells | Benign salivary gland neoplasm with oncocytic features with rare lymphocytes, consistent with Warthin tumor | No surgical follow-up | Ruling out carcinoma such as acinic cell carcinoma |
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|--------------------------------|--------------------------------------|----------------------------|----------------------------|
| IVb    | Fragments of basaloid epithelium; differential diagnosis include a salivary gland neoplasm with basaloid features versus metastatic papillary thyroid carcinoma | Negative for thyroglobulin | Salivary gland neoplasm with basaloid features | No surgical follow-up | Rule out metastatic papillary thyroid carcinoma (History of papillary thyroid carcinoma) |
| IVb    | Salivary gland neoplasm with focal squamous and glandular features | Positive for mucicarmine | Salivary gland neoplasm with focal squamous and mucinous features | High grade adenocarcinoma, consistent with salivary duct carcinoma | Confirming intracytoplasmic mucin |
| IVb    | Atypical epithelial cells, cannot exclude a low-grade salivary gland neoplasm | Positive for mucicarmine | Salivary gland neoplasm, low grade | Mucoepidermoid carcinoma | Confirming intracytoplasmic mucin |
| IVb    | Salivary gland neoplasm | Epithelial cells labeling with AE1/AE3 and myoepithelial labeling with P63 and SMA | Biphasic salivary gland neoplasm with epithelial and myoepithelial components | No surgical follow-up | Confirming epithelial and myoepithelial components |
| IVb    | Low grade salivary gland neoplasm with oncocytic features, however a secondary neoplasm such as melanoma cannot be entirely excluded | Negative for S100 | Low grade salivary gland neoplasm with oncocytic features | No surgical follow-up | Rule out melanoma |
| IVb    | Salivary gland neoplasm with basaloid features | Positive for cytokeratin; negative for CD45 | Salivary gland neoplasm with basaloid features | No surgical follow-up | Confirming the presence of epithelial cells |
| IVb    | Salivary gland neoplasm of uncertain malignant potential | P63 highlights myoepithelial cells | Salivary gland neoplasm with prominent myoepithelial cell population and scant stroma | No surgical follow-up | Identifying nature of the neoplastic cells |
| IVb    | Neoplasm with spindled and histiocytoid cells | Rare cells positive for S100; non-contributory MNF116; and HMB45 | Granular/histiocytoid neoplasm. The cytomorphologic differential diagnosis includes granular cell tumor, schwannoma and PEComa. Although this lesion is favored to be benign, a low-grade salivary gland neoplasm with oncocytic features, such as acinic cell carcinoma, cannot be completely ruled out | Granular cell tumor | To identify nature of the neoplastic cells |
| IVb    | Atypical spindle cell neoplasm | Negative for CK5/6; p63, and S100, Ki-67 less than 25% | Atypical spindle cell neoplasm, with focal basaloid epithelioid groups of uncertain significance in a myxoid background. These spindle cells may therefore not be myoepithelial, but only scant tissue is present for assessment. The findings are concerning for a malignant neoplasm, such as a low-grade sarcoma, but the spindle cells are not unequivocal for malignancy and the differential diagnosis includes a spectrum of tumors. | Myofibroblastic sarcoma | To identify nature of the neoplastic cells |
| MSRGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|-------|----------------------------------------|-------------------------------|----------------------------------|-----------------------------|-----------------------------|
| IVb   | Neoplasm with basaloid features        | Positive for P63; negative for CD31; CD34 and FLI-1 | Salivary gland neoplasm with basaloid features. The differential diagnosis includes recurrence of the patient’s prior basal cell carcinoma and primary salivary gland neoplasms (basal cell adenoma, basaloid squamous cell carcinoma, and adenoid cystic carcinoma) | No surgical follow-up | To identify nature of the neoplastic cells |
| IVb   | Salivary gland neoplasm with basaloid features | p63 highlights myoepithelial cells; negative for c-KIT | Salivary gland neoplasm with basaloid features. The differential diagnosis includes basal cell adenoma and pleomorphic adenoma; however, other low-grade basaloid neoplasms should also be considered in the differential. Lack of C-kit expression does not favor the possibility of adenoid cystic carcinoma to be considered in the differential. | Basal cell adenoma | To identify nature of the neoplastic cells |
| IVb   | Neoplasm with clear cells features | Positive for AE1/AE and CD10; focally positive for p63 and Calponin; negative for S100, DOG-1, c-KIT, RCC and PAX-8 | Neoplasm with clear cells features. Although CD10 positivity raises concern for metastatic renal cell carcinoma, the fact that the neoplastic cells are negative for RCC and PAX8 makes this possibility less likely although not entirely ruled out. CD10, is also a myoepithelial marker and together with focal positivity for p63 and calponin, raises the possibility of a primary salivary gland neoplasm of epithelial-myoepithelial origin | Metastatic renal cell carcinoma | To rule out acinic cell carcinoma and metastatic renal cell carcinoma in a patient with history of kidney malignancy status post nephrectomy |
| IVb   | Biphasic neoplasm with cytologic atypia, suspicious for malignancy | Positive for p63 and CK5/6; negative for S100 and mucicarmine | Biphasic neoplasm with cytologic atypia. The neoplasm shows epithelioid areas as well as spindled cells with some admixed matrix. The tumor is markedly cellular and has areas with prominent cytologic atypia. Focal areas of squamous differentiation are also seen. The differential diagnosis includes pleomorphic adenoma with atypia, carcinoma ex pleomorphic adenoma, basal cell adenoma and mucoepidermoid carcinoma. | Pleomorphic adenoma | To evaluate nature of the neoplastic cells |
| IVb   | Salivary gland neoplasm with oncocyes, lymphocytes, few squamous cells and debris | Positive for p63 and CK5/6; negative for mucicarmine | Low grade salivary gland neoplasm with oncocyes, lymphocytes, few squamous cells and debris. The differential diagnosis includes a Warthin tumor with squamous differentiation versus a low grade mucoepidermoid carcinoma with oncycotic change | Warthin tumor | To evaluate nature of the neoplastic cells |
| MSRSC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|-------|----------------------------------------|-------------------------------|---------------------------------|-----------------------------|-------------------------------|
| IVb   | Low grade salivary gland neoplasm      | Positive for AE1/AE3; negative for S100; Synaptophysin; chromogranin; and DOG-1 | Low grade salivary gland neoplasm | No surgical follow-up       | To evaluate nature of the neoplastic cells |
| IVb   | Salivary gland neoplasm with basaloid features | Negative for c-KIT | Myoepithelial rich salivary gland neoplasm. Based on morphology a diagnosis of cellular pleomorphic adenoma is favored. The other lesions to consider in the differential include monomorphic adenoma and myoepithelioma. Adenoid cystic carcinoma is less likely due to negative c-Kit stain. | Myoepithelioma | To rule out adenoid cystic carcinoma |
| IVb   | Low grade salivary gland neoplasm with focal squamous features and necrosis | Positive for S100 and p63; negative for c-KIT and mucicarmine | Low grade salivary gland neoplasm with focal squamous features and necrosis. The differential diagnosis includes epithelial-myoeplithelial salivary gland neoplasm including pleomorphic adenoma, epithelial/myoepithelial carcinoma and low grade mucopidermoid carcinoma | Pleomorphic adenoma | To identify nature of the neoplastic cells |
| IVb   | Salivary gland neoplasm | Negative for c-KIT | Salivary gland neoplasm, favor cellular pleomorphic adenoma. While a cellular pleomorphic adenoma or myoepithelioma is favored, a low-grade malignancy including myoepithelial carcinoma cannot be entirely excluded | Cellular pleomorphic adenoma | To rule out adenoid cystic carcinoma |
| IVb   | Neoplasm with focal clear cell features | Positive for AE1/AE3, p63, EMA (weak, focal), SMA, SMM-HC (weak), negative for desmin, S100, CD31 and HMB-45 | Neoplasm with focal clear cell features, favor pleomorphic adenoma, however a metastatic process cannot be excluded | Cellular pleomorphic adenoma | To identify nature of the neoplastic cells |
| IVb   | Salivary gland neoplasm | Negative for cytokeratin AE1/AE3, SMA, HMB-45 and Melan A and focally positive for S100 | Salivary gland neoplasm with abundant myoepithelial cells | Myoepithelioma | To identify the nature of cells |
| IVb   | Low grade salivary gland neoplasm | Positive for S100; negative for DOG-1, mammaglobin, and mucicarmine | Low grade salivary gland neoplasm, with eosinophilic vacuolated cytoplasm on cell block | Secretory carcinoma | To identify nature of the neoplastic cells |
| IVb   | Low grade salivary gland neoplasm, favor pleomorphic adenoma | Neoplastic epithelial cells positive for AE1/3 and C-KIT; Neoplastic myoepithelial cells positive for p63, AE1/3, S100, and calponin; Negative for synaptophysin | Low grade salivary gland neoplasm, favor pleomorphic adenoma | Recurrent esthesioneuroblastoma | To identify nature of the neoplastic cells |
| IVb   | Cellular epithelial neoplasm of salivary gland origin | Negative for mucicarmine | Cellular epithelial neoplasm of salivary gland origin | Metastatic carcinoma with neuroendocrine differentiation | Rule out mucoepidermoid carcinoma |
### TABLE 6  MSRSGC category IV: Suspicious for malignancy, cytology diagnosis, type of ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable)

| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|-------------------------------|-------------------------------------|-------------------------------|-----------------------------|
| V      | Suspicious for malignant neoplasm      | S100; HMB45; AE1/AE3, and CAM5.2 non-contributory due to limited cells | Suspicious for malignant neoplasm | Salivary duct carcinoma | To identify nature of the neoplastic cells |
| V      | Suspicious for malignant neoplasm      | Positive for AE1/AE3 and CAM5.2 | Suspicious for malignant neoplasm. The differential diagnosis includes acinic cell carcinoma and secretory carcinoma. | Salivary duct carcinoma | To identify nature of the neoplastic cells |
| V      | Atypical lymphoid infiltrate suspicious for lymphoproliferative disorder | Positive CD20 B cell lymphocytes; scattered CD3 positive T cells | Atypical lymphoid infiltrate suspicious for lymphoproliferative disorder | MALT lymphoma | To identify nature of the lymphocytes |
| V      | Suspicious for secretory carcinoma     | Positive for S100; negative for DOG-1, mammaglobin, and mucicarmine | Suspicious for secretory carcinoma | Secretory carcinoma | To identify nature of the neoplastic cells |

### TABLE 7  MSRSGC category IV: Malignant cytology diagnosis, type of ancillary studies performed on cell block, and surgical pathology diagnosis (if applicable)

| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|----------------------------------------|-------------------------------|-------------------------------------|-------------------------------|-----------------------------|
| VI     | Malignant neoplasm, favor carcinoma    | Positive for CK-7 and Mammaglobin Negative for CK20; p63; CK5/6; p40; S100; HMB45; TTF-1; Napsin A; Thyroglobulin; CDX2 and Mucicarmine | Malignant neoplasm, favor salivary duct carcinoma | Salivary duct carcinoma | To identify nature of the neoplastic cells |
| VI     | Neoplasm with neuroendocrine features  | Positive for Synaptophysin and chromogranin Negative for Actin | Metastatic neuroendocrine tumor | No surgical follow-up | To identify nature of the neoplastic cells |
| VI     | Adenocarcinoma                         | Positive for CK7: negative for CK20 | Adenocarcinoma | Salivary duct carcinoma | To identify nature of the neoplastic cells |
| VI     | Malignant neoplasm favor Metastatic melanoma | Positive for HMB 45 | Metastatic malignant melanoma | Metastatic malignant melanoma | Confirmed metastatic melanoma (history of melanoma) |
| VI     | Poorly differentiated non-small cell carcinoma | Negative for thyroglobulin | Poorly differentiated non-small cell carcinoma. Negative for papillary thyroid carcinoma | Acinic cell carcinoma | Ruling out papillary thyroid carcinoma (History of papillary thyroid carcinoma) |
| VI     | Mucoepidermoid carcinoma, low grade    | Positive mucicarmine stain | Mucoepidermoid carcinoma, low grade | No surgical follow-up | Detecting mucin |
| VI     | Mucoepidermoid carcinoma                | Positive mucicarmine stain | Mucoepidermoid carcinoma | Mucoepidermoid carcinoma | Detecting mucin |

(Continues)
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|-------|----------------------------------------|---------------------------------|-------------------------------------|-----------------------------|------------------------------|
| VI    | Malignant neoplasm                     | Positive for CD56, chromogranin, synaptophysin (weakly and focally), and CD56, negative for AE1/AE3, CD45, S100, p63, L-Actin, actin, MGN, and CD45 | Malignant neoplasm, favor metastatic oligodendroglioma | Metastatic oligodendroglioma | To confirm metastatic oligodendroglioma (history of oligodendroglioma) |
| VI    | Suspicious for lymphoma                 | Positive for CD20; Negative for CD10, BCL-6, CD5, CD23, and cyclin D1 | MALT lymphoma | No surgical follow-up | Confirming the diagnosis |
| VI    | High grade neoplasm, favor carcinoma    | Positive for cytokeratin; Negative for chromogranin, calcitonin, s-100, HMB-45, thyroglobulin, mucin | High grade carcinoma | High grade adenocarcinoma | Confirming the diagnosis of carcinoma |
| VI    | Malignant neoplasm with small cell features | Positive for CD56, chromogranin, and synaptophysin; negative for O13, CK20, CD3, CD10, CD20, CD45, Kappa and Lambda light chains; equivocal for AE1/AE3 | Small cell carcinoma | No surgical follow-up | Confirming the diagnosis |
| VI    | Acinic cell carcinoma                   | Positive for pan cytokeratin; Negative for GFAP, S100, smooth muscle actin, and mucicarmine | Acinic cell carcinoma | No surgical follow up | Confirming the diagnosis |
| VI    | Squamous cell carcinoma                 | Positive for P16 and HPV | HPV-related Squamous cell carcinoma | HPV-related squamous cell carcinoma | Detection of high-risk HPV |
| VI    | Involved by multiple myeloma            | Positive for CD138 and kappa; negative for lambda | Involved by multiple myeloma, kappa light chain restricted | No surgical follow-up | Confirming multiple myeloma (history of multiple myeloma) |
| VI    | Metastatic papillary thyroid carcinoma  | Positive for thyroglobulin | Metastatic papillary thyroid carcinoma | No surgical follow-up | Confirming Metastatic papillary thyroid carcinoma (history of papillary thyroid carcinoma) |
| VI    | Poorly differentiated malignant neoplasm | Positive for cytokeratin, AE1/AE3, CAM5.2, and mucicarmine; negative for S100, HMB45, and Melan A | Poorly differentiated adenocarcinoma | Invasive salivary duct carcinoma | To differentiate carcinoma from melanoma |
| VI    | Metastatic squamous cell carcinoma       | Positive for P63 and CAM5.2 | Metastatic squamous cell carcinoma | Poorly differentiated squamous cell carcinoma | To confirm the diagnosis |
| VI    | Squamous cell carcinoma                 | Positive for p16 and HPV ISH | HPV-related Squamous cell carcinoma | No surgical follow-up | Detection of high-risk HPV |
| MSRSGC          | FNA diagnosis without ancillary studies | Ancillary studies on cell block                                                                 | FNA diagnosis with ancillary studies  | Surgical pathology diagnosis                                      | Reason for ancillary studies                                      |
|----------------|----------------------------------------|---------------------------------------------------------------------------------------------|--------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------|
| VI             | Suspicious for large B cell lymphoma   | CD20 stains confluent sheets of large B; dimly positive for BCL-2, and lack CD5 and CD10. Ki-67 of 50%–60%. CD23, NKG3.1 are negative. | Large B cell lymphoma                | No surgical follow-up                                           | Confirming the diagnosis                                         |
| VI             | Poorly differentiated carcinoma with squamous features | Positive for p40                                                                           | Poorly differentiated squamous cell carcinoma | Invasive poorly differentiated squamous cell carcinoma          | Confirming the diagnosis                                         |
| VI             | High grade carcinoma                   | Positive for AR; negative for S100; Mammaglobin; HER2/Neu; and mucin stain                    | High grade carcinoma, favor salivary duct carcinoma | Salivary duct carcinoma, micropapillary pattern                | Confirming the diagnosis                                         |
| VI             | Poorly differentiated malignant neoplasm | Positive for AE1/AE3; p63 and p40; negative for SOX10; MART-1; Melan A; S100; CK7; CK20; CK5/6; p16; Mucin stain | Poorly differentiated squamous cell carcinoma | Poorly differentiated squamous cell carcinoma                   | To evaluate nature of the neoplastic cells                        |
| VI             | Salivary gland neoplasm, most consistent with secretory carcinoma | Positive for CK19; Mammaglobin; and S100; negative for p63; DOG-1, and PAS-D                | Salivary gland neoplasm, most consistent with secretory carcinoma | No surgical follow-up                                           | Confirming the diagnosis                                         |
| VI             | Suspicious for secretory carcinoma     | Positive for CK7; CK8/18; SMA; Mammaglobin; and S100; negative for CK20; Ber-Ep4; CK5/6 and p63 | Secretory carcinoma                  | No surgical follow-up                                           | Confirming the diagnosis                                         |
| VI             | Low grade neoplasm. The differential diagnosis includes acinic cell carcinoma and less likely a metastatic process | Positive for CK7 and Vimentin; negative for CK20; P63 and CD10                              | Acinic cell carcinoma                 | No surgical follow-up                                           | To exclude metastatic carcinoma                                  |
| VI             | Malignant neoplasm, favor sarcoma      | Positive for Myogenin; MyoD1; desmin; and SMA                                               | Alveolar rhabdomyosarcoma             | No surgical follow-up                                           | To confirm the diagnosis                                          |
| VI             | Metastatic melanoma                    | Positive for S100                                                                            | Metastatic melanoma                  | No surgical follow-up                                           | To confirm the diagnosis                                          |
| VI             | Poorly differentiated neoplasm with necrosis, suggestive of metastatic glioblastoma | Positive for GFAP; focally positive for AE1/AE3; negative for S100                            | Poorly differentiated neoplasm with necrosis, consistent with metastatic glioblastoma | No surgical follow-up                                           | To confirm the diagnosis                                          |
| VI             | Poorly differentiated malignant neoplasm with necrosis | Positive for AE1/AE3 and CK7; focally positive for GCDFP; negative for Melan A; S100; CDX-2; TTF-1; Mucin stain | Poorly differentiated carcinoma with necrosis | No surgical follow-up                                           | To evaluate nature of the neoplastic cells and rule out a metastatic process |

(Continues)
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|--------------------------------------|-------------------------------|------------------------------------|-----------------------------|-----------------------------|
| VI     | Squamous cell carcinoma              | Negative for p16; HPV ISH     | Squamous cell carcinoma, non-HPV related | No surgical follow-up       | Rule out HPV                |
| VI     | Suspicious for Large B cell lymphoma | Positive for CD20; CD10 and BCL-6; negative for BCL-2; CD45; CD30; MUM1; EBV ISH, few T cells positive for CD3; CD43; BCL-2 | Large B cell lymphoma       | No surgical follow-up       | Confirming large B cell lymphoma |
| VI     | Malignant neoplasm suspicious for Metastatic Merkel cell carcinoma | Positive for synaptophysin and CK20; negative for chromogranin | Metastatic Merkel cell carcinoma | Metastatic Merkel cell carcinoma | Confirming the diagnosis |
| VI     | Suspicious for mucoepidermoid carcinoma | Positive PAS stain | Mucoepidermoid carcinoma | Mucoepidermoid carcinoma | Detection of mucin |
| VI     | Squamous cell carcinoma              | Positive for p16; negative for Mucin stain | p16-positive Squamous cell carcinoma | No surgical follow-up       | To evaluate p16 and detect mucin |
| VI     | Squamous cell carcinoma              | Positive for p40 and p16 | p16-positive Squamous cell carcinoma | No surgical follow-up       | To confirm the diagnosis and detect p16 |
| VI     | Squamous cell carcinoma              | p16 positive                   | p16-positive Squamous cell carcinoma | No surgical follow-up       | To detect p16                |
| VI     | Atypical lymphoid cells concerning for Hodgkin lymphoma | Positive for CD30 and CD15 | Hodgkin lymphoma | Classical Hodgkin lymphoma type, EBV +, recurrent, post-transplant | To confirm a diagnosis |
| VI     | Carcinoma                            | AR equivocal; negative for TTF-1 and NapsinA | Salivary duct carcinoma | Salivary duct carcinoma | To confirm a diagnosis and rule out a metastatic process in a patient with history of lung adenocarcinoma |
| VI     | Atypical lymphoid tissue             | Positive for CD45, CD20, and vimentin, negative for CD30; AE1/AE3; CAM5.2; and S100 | Atypical lymphoid tissue consistent with lymphoma | Follicular lymphoma, grade 3B | To evaluate nature of the neoplastic cells |
| VI     | Carcinoma                            | Positive for AE1/AE3 and AR; focally positive for mammaglobin and p63; negative for S100 | Salivary duct carcinoma | No surgical follow-up       | To confirm a diagnosis |
| VI     | Salivary gland neoplasm with features suggestive of acinic cell carcinoma | Positive for DOG-1; negative for mammaglobin; p63 and S100 | Acinic cell carcinoma | Acinic cell carcinoma | To confirm a definitive diagnosis |
| VI     | Poorly differentiated carcinoma      | Focally positive for P40; CK5/6 | Poorly differentiated squamous cell carcinoma | Poorly differentiated squamous cell carcinoma | Confirming the diagnosis |
| VI     | Poorly differentiated malignant neoplasm | Positive for CAM5.2 Negative for CK20; chromogranin; synaptophysin; TTF-1; CD20 and CD5 | Poorly differentiated carcinoma | No surgical follow-up       | To evaluate nature of the neoplastic cells |
| MSRSGC | FNA diagnosis without ancillary studies | Ancillary studies on cell block | FNA diagnosis with ancillary studies | Surgical pathology diagnosis | Reason for ancillary studies |
|--------|--------------------------------------|---------------------------------|------------------------------------|----------------------------|-----------------------------|
| VI     | Malignant salivary gland neoplasm    | Negative for AR and HER2/Neu    | Malignant salivary gland neoplasm  | Salivary Duct carcinoma    | To confirm a diagnosis      |
| VI     | Poorly differentiated malignant neoplasm | Positive for AE1/AE3         | Poorly differentiated carcinoma   | No surgical follow-up      | To confirm carcinoma        |
| VI     | Poorly differentiated carcinoma with squamous differentiation | Positive for AE1/AE3; CK19; p63; EGFR; focally positive for GATA3; negative for AR and mucicarmin| Poorly differentiated carcinoma with squamous differentiation | Metastatic squamous cell carcinoma | To confirm carcinoma and squamous differentiation |
| VI     | Neoplasm with spindled and epithelioid cells present | Positive for AE1/AE3; vimentin; S100 and CD10; negative for RCC; CK7; SMA; TTF1 | Neoplasm with spindled and epithelioid cells present | No surgical follow-up      | To evaluate nature of the neoplastic cells and rule out a metastatic process |
| VI     | Malignant neoplasm suggestive of Merkel cell carcinoma | Positive for AE1/AE3; CK20 and PAX5 | Merkel cell carcinoma | No surgical follow-up      | To confirm metastasis of patient's known Merkel cell carcinoma |
| VI     | Poorly differentiated malignant neoplasm | Positive for AE1/AE3; negative for S100 | Poorly differentiated malignant neoplasm, favor carcinoma | Salivary duct adenocarcinoma with in situ component | To confirm carcinoma |
| VI     | Poorly differentiated malignant neoplasm | Positive for myogenin, desmin, and AE1/AE3; negative for CAM5.2 | Poorly differentiated malignant neoplasm consistent with patient's known malignant neoplasm | No surgical follow-up      | To confirm recurrence or metastasis of patient's known malignant neoplasm |
| VI     | Poorly differentiated malignant neoplasm with spindle and epithelioid features | positive for p63 and CAM5.2 (weak focal); negative for AE1/AE3, S100, HMB45, MiTF and Melan-A | Poorly differentiated malignant neoplasm with spindle and epithelioid features | Metastatic melanoma        | To evaluate nature of the neoplastic cells |
| VI     | Poorly differentiated squamous cell carcinoma | Negative for p16 | Poorly differentiated squamous cell carcinoma, P16 negative | Metastatic squamous cell carcinoma | To exclude HPV related carcinoma |
| VI     | Poorly differentiated malignant neoplasm | Positive for AE1/AE3; negative for S100; Melan A | Poorly differentiated carcinoma | No surgical follow-up      | To confirm carcinoma and ruling out melanoma |
| VI     | Poorly differentiated carcinoma with neuroendocrine features | Positive for AE1/AE3; CK20; chromogranin and synaptophysin; negative for CK7; S100; HMB45; Melan-A and TTF-1 | Poorly differentiated carcinoma with neuroendocrine features | Metastatic poorly differentiated carcinoma | To confirm carcinoma and ruling out melanoma |
| VI     | Metastatic melanoma | Positive for Melan A and HMB45, negative for AE1/AE3 and S100 | Metastatic melanoma | Melanoma                   | To confirm metastatic melanoma |
| VI     | Metastatic melanoma | Positive for S100; HMB45; Melan A; negative for AE1/AE3 | Metastatic melanoma | Melanoma                   | To confirm metastatic melanoma |
| VI     | Poorly differentiated carcinoma with vacuolated and pleomorphic cells | Positive for AE1/AE3, CK20 | Poorly differentiated carcinoma with vacuolated and pleomorphic cells | Poorly differentiated carcinoma | To confirm diagnosis of carcinoma and excluding a metastatic process |
histiocytes was diagnosed as Rosai-Dorfman disease on surgical follow-up.

4 | DISCUSSION

In this study we evaluated the utility of ancillary studies including IHC and histochemical staining, and ISH performed on FNA cell blocks in diagnosis of salivary gland lesions classified according to MSRSGC, and the impact on clinical decision-making. Ancillary studies applied on SG FNA such as molecular studies, or FISH were not included in this study. The low number of cases in this study is an evidence that cell blocks either are not routinely prepared for all SG FNA cases or they may not contain sufficient material for subsequent studies. Therefore, this study and its finding presents a small number of cases that contained sufficient material for ancillary studies.

The amorphous matrix in the background posed diagnostic difficulties, particularly in cystic and hypocellular specimens. Mucicarmine stain and thyroglobulin were used to highlight mucin and colloid in two cystic cases, respectively (Table 2).

The presence of inflammatory cells, epithelioid histocytes and granulomatous inflammation triggered the initial pathologists to investigate an underlying infectious process. Gram stain, GMS stain, Zeihl Neelsen stain, Warthin–Starry stain, Brown Hopp’s stain, and spirochete immunostains were utilized in these cases. Although a negative stain cannot exclude an infectious process, a positive stain detecting microorganisms confirms an infectious process. These stains were utilized more often in non-neoplastic cases (Tables 1 and 2). The presence of atypical lymphocytes on aspirated material can be due to either reactive changes or a lymphoproliferative disorder. Flow cytometry studies can be requested on aspirated material if there is on-site evaluation for specimen adequacy. Immunostains used for detection of lymphoproliferative/hematopoietic disorders such as CD3, and CD20 were commonly utilized to rule out monoclonal proliferation of T cell or B cell lymphocytes, respectively. A selective panel of immunostains along with cytomorphology confirmed the diagnosis of lymphoma in several cases (Table 7). Recurrence of Hodgkin lymphoma was confirmed by positive CD15 and CD30 immunostains in a post-transplant patient. Plasma cell markers such as CD138, kappa and lambda were used to differentiate polyclonal from monoclonal plasma cell proliferations. These diagnostic or confirmatory immunostains for detection of lymphoproliferative or hematopoietic disorders improved the MSRSGC by separating malignant cases from reactive cases and decreasing the number of cases in indeterminate categories (atypical or suspicious). The sampling issue was a contributing factor to cytology diagnosis of indeterminate category in a subset of cases. In cyst content cases with epithelioid, poorly preserved or atypical macrophages, histiocytic markers, such as CD68, can confirm their identity and help prevent an atypical diagnosis. IgG-related sialadenitis was diagnosed in a few cases by applying IgG4 immunostain in those cases that were suspicious for IgG4-related chronic sialadenitis.

Clinical history of prior malignancy along with cytomorphologic features suspicious for a recurrence or a metastatic process played a key role in selecting immunostains in a subset of patients. For example, cytokeratin AE1/AE3, CK20, and PAX5 immunostains were ordered on aspirated material from parotid gland of a patient previously diagnosed for Merkel cell carcinoma to confirm a metastatic process or TTF-1 and Napsin-A were reviewed to rule out a metastatic lung adenocarcinoma. The material in cell block of cases with confirmed recurrence or metastatic disease can be further utilized for molecular testing, which can be explored in a future study. Additionally, p16 and HPV in situ hybridization were utilized to detect HPV-related or p16 positive squamous-cell carcinoma cases, which has prognostic implication compared to its HPV-negative or p16-negative counterpart.

Immunostains and mucicarmine stain were used to confirm a diagnosis of a salivary gland neoplasm in a subset of cases. For instance DOG-1 was used to confirm a case of acinic cell carcinoma. Mammmaglobin was helpful in diagnosis of secretory carcinoma.

| TABLE 7 | Ancillary studies on cell block | Reason for ancillary studies |
|---------|-------------------------------|-----------------------------|
| VI      | Melanoma                      | To confirm metastatic melanoma |
| VI      | Squamous cell carcinoma       | Excluding HPV related carcinoma |
| VI      | Poorly differentiated carcinoma with neuroendocrine features | To confirm the diagnosis |

Abbreviations: Ca, carcinoma; IHC, immunohistochemical stains; ISH, in situ hybridization; MALT, marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; SI, surgical intervention.
cases.12 Androgen receptor immunostain was positive in salivary duct carcinoma, while p63 was negative.13,14 However, unusual or uncommon cytomorphic findings of salivary gland neoplasms guided the pathologists to select immunostains based on those findings. In cases with atypical or poorly-preserved epithelial fragments, p40 and p63 highlighted squamous differentiation. Cytokeratin AE1/AE3 and CAM5.2 confirmed the epithelial origin of neoplastic cells in several cases. Pleomorphic adenoma is the most common salivary gland neoplasm, which is usually diagnosed on routine stains. However, immunostains and mucicarmine stain were utilized in several cases of pleomorphic adenomas due to their unusual cytomorphic presentations such as focal clear cell features or necrosis. Myoepithelial cells of pleomorphic adenomas can create diagnostic challenges when present in high proportion of cells or when presenting with variable morphology such as spindle cell morphology. Cellular pleomorphic adenomas presented with basaloid features were evaluated with p63 and c-KIT markers to rule out adenoid cystic carcinoma. Myoepithelial cells in pleomorphic adenoma are immunoreactive for p40 and p63 and negative for c-KIT, while adenoid cystic carcinoma is immunoreactive for c-KIT and negative for p40 and p63.15 Mucicarmine stain was used to evaluate cells with intracellular mucin such as those seen in mucoepidermoid carcinoma cases. Metaplastic changes associated with necrosis or atypia raised the possibility of a malignant process in several cases otherwise appearing benign. Squamous metaplasia presented as necrotic keratinized cells and keratin as abundant eosinophilic necrotic and mummified material, which were confirmed by AE1/AE3 and amyloid stain in a case of oncocytoma. Squamous metaplasia and numerous foamy macrophages in a Warthin tumor raised the possibility of a low grade mucoepidermoid carcinoma. Mucin stain was negative and p63 highlighted squamous cells. Of note, all cases with unusual presentations which were accompanied with ancillary studies, were reviewed by another cytopathologist with expertise in salivary gland cytology in all three institutions. Based on these findings, it is evident that ancillary studies may reduce or refine the number of atypical diagnoses to more definitive diagnostic categories of MSRSGC.

5 | CONCLUSION

This multi-institutional study demonstrates the diagnostic utility of ancillary studies including immunohistochemistry, histochemistry, in situ hybridization, and stains for infectious agents in cell blocks prepared from aspirated salivary gland lesions in a very small subset of cases. Ancillary studies performed on cell blocks assisted to further characterize: 1) the atypical lymphocytes, neoplastic cells or their origin, 2) the matrix in the background (mucin vs. colloid), 3) unusual presentation of neoplasms and metaplastic changes, and 4) to rule out a metastatic process of a known malignancy. Ancillary studies performed on SG FNA cell blocks with sufficient material can improve the diagnostic yield by further characterization of the atypical/neoplastic cells, particularly in MSRSGC categories IVa–VI. Ancillary studies should be used judiciously and case-based to improve diagnosis in challenging cases. The findings of this study are more case-based and future studies with larger cohorts are required to evaluate the comprehensive role of ancillary studies, including molecular studies and FISH on cell blocks, prepared from SG FNA specimens.

CONFLICT OF INTEREST

No conflict of interest declared.

AUTHOR CONTRIBUTIONS

Background research, drafting of the manuscript, conception of the idea, and critical revision: Seena Tabibi. Background research, drafting of the manuscript, conception of the idea, and critical revision: Matthew Gabrielson. Background research, drafting of the manuscript, conception of the idea, and critical revision: Carla Saoud. Background research, drafting of the manuscript, conception of the idea, and critical revision: Katelynn Davis. Background research, drafting of the manuscript, conception of the idea, and critical revision: Sintawat Wangsiricharoen. Data collection and editing: Ryan Lu. Data collection and editing: Isabella Tondi Resta. Data collection, and critical revision of the manuscript for important intellectual content: Kartik Viswanathan. Collation of cases and critical revision of the manuscript for important intellectual content: William C. Faquin. Collation of cases and critical revision of the manuscript for important intellectual content: Zubair Baloch. Conception of the idea for the manuscript and its design and coordination, collation of cases, visualization and critical revision of the manuscript for important intellectual content: Zahra Maleki. All authors have read and approved the final manuscript and have declared that they qualify for authorship.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

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REFERENCES

1. Behaeghe M, Vander Poorten V, Hermans R, Politis C, Weynand B, Hauben E. The Milan system for reporting salivary gland cytopathology: single center experience with cell blocks. Diagn Cytopathol. 2020;48:972-978.
2. Layfield LJ, Glasgow BJ. Diagnosis of salivary gland tumors by fine-needle aspiration cytology: a review of clinical utility and pitfalls. Diagn Cytopathol. 1991;7:267-272.
3. Eytan DF, Yin LX, Maleki Z, et al. Utility of preoperative fine needle aspiration in parotid gland lesions. Laryngoscope. 2018;128:398-402.
4. Allison DB, Smith AP, An D, et al. Assessing the diagnostic accuracy for pleomorphic adenoma and Warthin tumor by employing the Milan System for Reporting Salivary Gland Cytopathology: An international, multi-institutional study. Cancer Cytopathol. 2021;129:43-52.
5. Schmidt RL, Hall BJ, Wilson AR, 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. 2011;136:45-59.
6. Dubucs C, Basset C, D’Aure D, Courtaud-Saidi M, Evrard SM. A 4-year retrospective analysis of salivary gland cytopathology using
the Milan System for Reporting Salivary Gland Cytology And Ancillary studies. Cancers (Basel). 2019;11(12):1912.

7. Salehi S, Maleki Z. Diagnostic challenges and problem cases in salivary gland cytology: a 20-year experience. Cancer Cytopathol. 2018;126:101-111.

8. Griffith CC, Pai RK, Schneider F, et al. Salivary gland tumor fine-needle aspiration cytology: a proposal for a risk stratification classification. Am J Clin Pathol. 2015;143:839-853.

9. Faquin WC, Rossi ED, Baloch Z, et al., eds. The Milan System for Reporting Salivary Gland Cytopathology. Springer International Publishing; 2018.

10. Ribeiro EA, Maleki Z. p16 immunostaining in cytology specimens: its application, expression, interpretation, and challenges. J Am Soc Cytopathol. 2021;10:414-422.

11. Jo VY, Krane JF. Ancillary testing in salivary gland cytology: a practical guide. Cancer Cytopathol. 2018;126(Suppl 8):627-642.

12. Wang H, Fundakowski C, Khurana JS, Jhala N. Fine-needle aspiration biopsy of salivary gland lesions. Arch Pathol Lab Med. 2015;139:1491-1497.

13. Point du Jour K, Griffith CC. The role of ancillary techniques in salivary gland cytopathology specimens. Acta Cytol. 2020;64:92-102.

14. Nakaguro M, Tada Y, Faquin WC, Sadow PM, Wirth LJ, Nagao T. Salivary duct carcinoma: updates in histology, cytology, molecular biology, and treatment. Cancer Cytopathol. 2020;128:693-703.

15. Griffith CC, Siddiqui MT, Schmitt AC. Ancillary testing strategies in salivary gland aspiration cytology: a practical pattern-based approach. Diagn Cytopathol. 2017;45:808-819.

How to cite this article: Tabibi S, Gabrielson M, Saoud C, et al. Ancillary studies on cell blocks from fine needle aspiration specimens of salivary gland lesions: a multi-institutional study. Diagnostic Cytopathology. 2022;50(5):235-252.
doi:10.1002/dc.24939