Original Article

Secretory carcinoma of the salivary gland, a rare entity: An international multi-institutional study

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BACKGROUND: Secretory carcinoma (SC) of the salivary gland is a rare entity with limited published literature on cytomorphology. The authors present the largest cohort to date of SC fine-needle aspiration (FNA) cases. **METHODS:** FNA cases of histologically confirmed SC were retrospectively retrieved from 12 academic institutions in the United States, Italy, Finland, and Brazil. The collated data included patient demographics, imaging findings, cytopathologic diagnoses according to the Milan System for Reporting Salivary Gland Cytopathology, cytomorphologic characteristics, and immunohistochemical/molecular profiles. **RESULTS:** In total, 40 SCs were identified (male-to-female ratio, 14:26) in patients with a mean age of 52 years (age range, 13-80 years). Ultrasound imagining revealed a hypoechoic, ovoid, poorly defined, or lobulated mass. The most common primary site was the parotid gland (30 of 40 tumors). Regional lymph node metastasis (9 patients) and distant metastasis (4 patients; brain, liver, lungs, and mediastinum) were noted. Two patients died of disease. FNA smears were cellular and demonstrated mainly large, round cells with intracytoplasmic vacuoles or granules and round-to-oval nuclei with smooth nuclear contour, minimal irregularities, and prominent nucleoli arranged predominantly in clusters, papillary formations, and single cells. The background was variable and contained inflammatory cells, mucin, or proteinaceous material. The diagnoses were malignant (19 of 38 tumors; 50%), suspicious for malignancy (10 of 38 tumors; 26%), salivary gland neoplasm of uncertain malignant potential (7 of 38 tumors; 18%), and atypia of undetermined significance (2 of 38 tumors; 6%) according to the Milan System for Reporting Salivary Gland Cytopathology. Two malignant cases (2 of 40 tumors; 5%) were metastases. The neoplastic cells were immunoreactive for S100 (23 of 24 tumors), mammaglobin (18 of 18 tumors), GATA-3 (13 of 13 tumors), AE1/AE3 (7 of 7 tumors), and vimentin (6 of 6 tumors). *ETV6-NTRK3* fusion was detected in 32 of 33 tumors by fluorescence in situ hybridization (n = 32) and next-generation sequencing (n = 1). **CONCLUSIONS:** Familiarity with cytomorphologic features and the immunohistochemical/molecular profile of SC can enhance diagnostic accuracy. ***Cancer Cytopathol* 2022;0:1-11**.

KEY WORDS: cytology; *ETV6-NTRK3*; fine-needle aspiration; mammaglobin; mammary analogue secretory carcinoma; Milan System for Reporting Salivary Gland Cytopathology; salivary gland; secretory carcinoma.

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INTRODUCTION

Secretory carcinoma (SC) of the salivary glands was first defined in 2010. Previously, it was classified as a zymogen-poor variant of acinic cell carcinoma (AciCC). Identification of the pathognomonic fusion gene *ETV6-NTRK3*, because it harbored the translocation t(12;15)(p13;q25), established SC as a distinct entity from AciCC. Salivary gland SC is morphologically, immunohistochemically, and genetically similar to SC of the breast.

Clinically, SC is a low-grade malignancy of the salivary glands with a typically indolent course and a good response to surgical resection.[1] Cases may be widely distributed from childhood to the elderly; however, SC commonly occurs in adults. SC predominately affects the parotid gland and, less frequently, the submandibular gland and minor salivary glands throughout the head and neck and the airway system.[2,3] Metastases to regional cervical lymph nodes are uncommon but have been reported in one-fifth of cases. Distant metastases are rare. Metastasis of SC can be associated with high-grade transformation and a poor prognosis. Mortality from SC is rare.[4]

SC demonstrates various cytomorphologic, macroscopic, and histologic features that overlap with other tumors, including AciCC, as well as other benign neoplasms and nonneoplastic conditions of the salivary gland.[5,6] In addition, the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was published in 2018 and has been increasingly accepted by different pathologists and institutions to the point of becoming a universal language of reporting salivary gland cytology.[7] The MSRSGC is a 6-tiered diagnostic category system with associated risk stratification and management for each category. SC can pose diagnostic challenges on cytology specimens and subsequently can affect how specimens are categorized in the MSRSGC. Without any ancillary studies, a definitive diagnosis may be reached only by histologic examination of the excised tumor.[5] For the current study, we collated 40 fine-needle aspiration (FNA) cases of salivary SC from 12 academic institutions and analyzed the cytomorphologic findings, MSRSGC category, and histochemical and immunoprofile of both the FNA biopsy and the resection specimen, with correlation to molecular studies, radiology, and patient demographics, when available.

MATERIALS AND METHODS

Institutional Review Board approval for a retrospective search of the electronic health records for cases of SC of the salivary gland was obtained from 12 hospitals across the United States, Europe, and South America. Eight institutions were located in the United States: Johns Hopkins Hospital (Baltimore, Maryland), Massachusetts General Hospital (Boston, Massachusetts), Brigham and Women's Hospital (Boston, Massachusetts), Virginia Commonwealth University (Richmond, Virginia), the Perelman School of Medicine at the University of Pennsylvania (Philadelphia, Pennsylvania), the University of Pittsburgh (Pittsburgh, Pennsylvania), Moffitt Cancer Center (Tampa, Florida), and the Robert Wood Johnson School of Medicine (New Brunswick, New Jersey). Three institutions were located in Europe: Catholic University of the Sacred Heart (Rome, Italy), University Hospitals (Milan, Italy), and Fimlab Laboratories (Tampere, Finland). One institution was located in South America: Federation University of Sao Paulo (Sao Paulo, Brazil).

The pathology information systems of these 12 academic institutions were searched for cases of SC that had undergone FNA biopsy during the workup. For search query purposes, both *secretory carcinoma* and *mammary analogue secretory carcinoma* were included to account for changes in diagnostic terminology over time.

Patient demographic and clinical data were collected from electronic health records, including age at the time of diagnosis, sex, radiologic findings (salivary gland mass and other associated findings), anatomic site of FNA, mode of FNA (palpation or ultrasound guidance), specimen assessment for adequacy by rapid on-site evaluation, number of passes, known occurrence of metastasis, and any disease-related deaths. Various methods of specimen preparation (including conventional smears, liquid-based cytology [LBC] [ThinPrep 5000 method; Hologic Inc], cytospins, and cell blocks) and staining (including Diff-Quik staining on air-dried slides for rapid on-site evaluation and Papanicolaou staining on conventional slides fixed with an alcohol-based fixative) were used according to the different protocols and preferences of each institution. Detailed information regarding the cytomorphology of FNA specimens was also collated, including cellularity, architecture, cellular morphology, nuclear and cytoplasmic features, the presence or absence of mitotic figures, the presence or absence of inflammatory cells, and background characteristics. Ancillary studies, including histochemical and immunohistochemical studies performed on FNA smears, cell blocks, core biopsies, and

resection specimens, were tabulated. Available molecular study results, including fluorescence in situ hybridization (FISH) studies and next-generation sequencing, also were collected. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) diagnostic category, which was used for FNA diagnosis or was applied retrospectively in cases obtained before MSRSGC implementation, also was recorded.

All authors provided the information for their own institutional cases, including the related cytology and histology review. No central review was performed. The deidentified data were collected and were further reviewed and analyzed by 2 pathologists (A.W. and Z.M.).

RESULTS

The demographic and clinical parameters of 40 cases of SC biopsied using FNA are summarized in Table 1. The patient cohort comprised of 26 females and 14 males with a mean age at diagnosis of 52 years (age range, 13-80 years). The clinical presentation of the tumor was recorded as salivary gland mass, neck mass or lump, swelling, and neck mass with discomfort (1 case).

The FNA biopsy sites were labeled as salivary gland (34 of 40 biopsies), neck (4 of 40 biopsies), and oral (2 of 40 biopsies). The parotid gland (30 of 34 biopsies) was the most common salivary gland biopsied with FNA. One FNA case (1 of 4 biopsies) from the neck was a primary tumor originating from tracheal minor salivary glands, which showed direct extension into the thyroid gland and subsequent nodal metastasis.

FNA was performed under ultrasound guidance in 31 of 40 cases. The number of FNA passes ranged from 1 to 6 (average, 3 passes). On ultrasound, tumors were hypoechoic and ovoid in shape, having either poorly defined or lobulated borders. Five of 40 FNAs were performed with palpation guidance. The FNA method used was not available in 4 of 40 cases. The majority of metastatic and primary SCs were identified as solid and homogenous on computed tomography scans. On imaging, 1 of 40 cases displayed cystic changes, 1 on a computed tomography scan and the other on ultrasound (an intraoral buccal cyst), which was corroborated with the cytomorphology. Neck metastases showed positron emission tomography avidity in 1 of 1 case. The findings described above are summarized in Table 1. The slide preparation method was available in 38 of 40 cases, including smear only (13 of 38 cases); cytospin only (1 of 38 cases); smear and cell block (9 of 38 cases); LBC and cell block (2 of 38 cases); cytospin and cell block (1 of 38 cases); smear, LBC, and cell block (5 of 38 cases); and smear and LBC (7 of 38 cases).

TABLE 1. Summary of Clinical and Demographic Parameters of Patients From Nine Academic Institutions With Secretory Carcinoma Fine-Needle Aspiration Biopsies

Clinical Information	No. of Patients
Demographics	
Males	14
Females	26
Age: Mean (range), y	52.1 (13-80)
Anatomic site of FNA biopsy	
Oral	2
Neck	4
Parotid	30
Submandibular	2
Submental	2
Imaging findings summary	
Ultrasound	Hypoechoic, ovoid, poorly defined or lobulated borders
Computed tomography	Frequently solid and homogeneous; infrequently cystic (n = 1)
Positron emission tomography (PET)	PET-positive lymph nodes
Cases with metastasis during clinical course (n = 11)	Regional cervical lymph nodes only (7), 2 with extranodal extension
	Regional cervical lymph nodes and brain metastasis (1)
	Regional cervical lymph nodes, pterygoid recurrence and lung metastasis (1)
	Liver metastasis (1)
	Lungs and mediastinum (1)
Death related to secretory carcinoma (n = 2)	1 (13 months after diagnosis)
	1 (12 years after diagnosis)

Abbreviation: FNA, fine-needle aspiration.

The detailed cytomorphologic features were available in 32 of 40 cases, and most cases displayed a mixture of features. The majority of FNA biopsies of SC demonstrated high cellularity (27 of 32 cases) with cellular crowding (27 of 32 cases). The cells were arranged in clusters (21 cases), papillary and micropapillary formations (13 cases), single cells (12 cases), and sheets (6 cases). Less common features were acinar formation (2 cases), tubular formation (1 cases), transgressing vessels (2 cases), fibrovascular cores (1 case), microcystic aggregation (1 case), and nuclei with stripped cytoplasm (2 cases). Although the cytomorphologic findings were recorded for each case, and not for each slide, it appeared that large tissue fragments and sheets of malignant cells were observed more commonly in smears, and small clusters and single cells were observed more commonly in liquid-based–prepared slides. Papillary features were observed both in smears and in liquid-based–prepared slides. The cells were

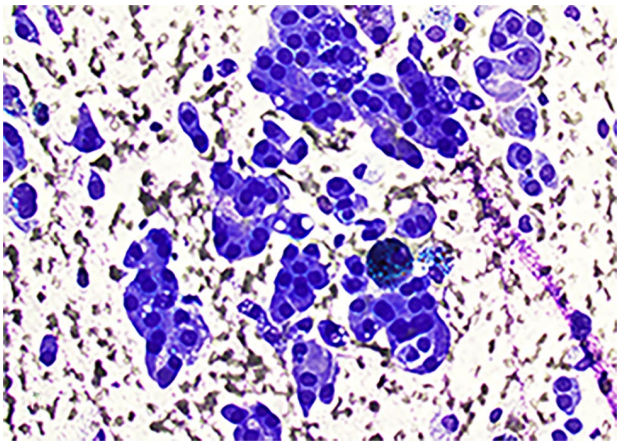

Figure 1. Clusters of relatively bland neoplastic cells exhibit round nuclei and moderate-to-abundant cytoplasm (original magnification ×200, Diff-Quik stain).

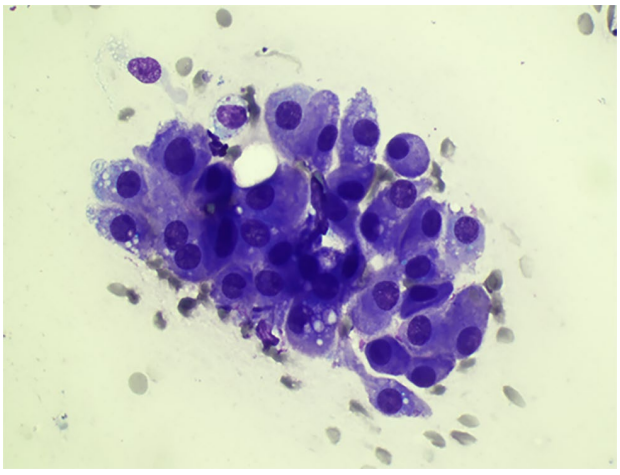

Figure 2. A cluster of loosely cohesive neoplastic cells is shown with round nuclei and moderate-to-abundant, focally vacuolated cytoplasm (original magnification ×400, Diff-Quik stain).

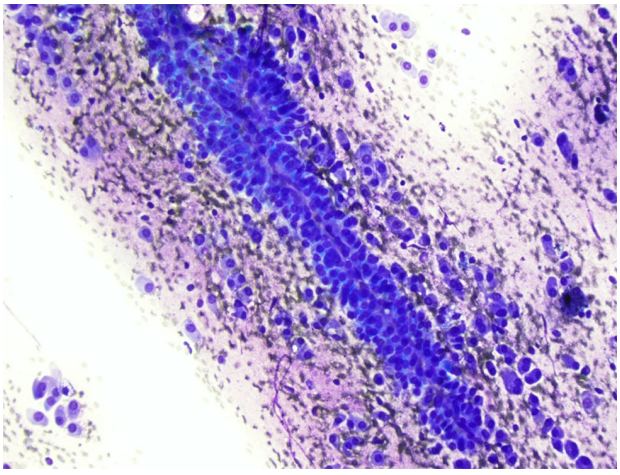

Figure 3. Neoplastic cells are arranged as papillary structures with fine fibrovascular cores, small clusters, and single cells in a loose matrix (original magnification ×200, Diff-Quik stain).

predominantly medium-to-large and round-to-polygonal (28 of 32 cases; 87.5%).

Oncocytic cells (5 cases) with mild atypia (6 cases), epithelioid cells (5 cases), clear cells (2 cases), plasmacytoid cells (1 case), small acinar-type cells (1 case), small duct-type cells (1 case), hobnail cells (1 case), short spindle cells (1 case), and cyst-lining–type cells (1 case) were less commonly described features. The cytoplasm was moderate-to-abundant in most cases (29 of 32; 90.6%) and appeared eosinophilic on Diff-Quik staining and cell blocks. The cytoplasm was vacuolated in 23 of 32 cases (71.8%), including 16 with large vacuoles, 6 with small vacuoles, and 1 with both microvacuoles and macrovacuoles, with occasional signet-like macrovacuoles (Figs. 1 and 2). Granular cytoplasm with or without vacuoles was observed in 11 of 32 cases (34.3%). Metachromatic, bright, intracytoplasmic globules were noted on Diff-Quik–stained slides in 3 cases. The nuclei were predominantly medium-to-large and round-to-oval (32 of 32 cases), with prominent small-to-large nucleoli (22 of 32 cases) and, in most cases, were eccentrically located (18 of 32 cases). Occasional binucleation (2 cases) and rare nuclear inclusions (2 cases) were other features observed. Mild nuclear atypia was seen in 5 cases. The nuclear contours were smooth with focal irregularities. The chromatin was variable, from fine-to-coarse and pale-to-hyperchromatic, whenever data were available. Rare mitotic figures were present in 7 of 32 cases (21.8%). Scattered lymphocytes (10 of 32 cases), neutrophils (3 of 32 cases), macrophages (some hemosiderin-laden; 5 of 32 cases), and multinucleated giant cells (4 of 32 cases) were present in the background. The background was mucinous (8 cases), with bubbly secretions (2 cases), granular with amorphous proteinases material (9 cases), and exhibited metachromatic globules and fibrillary matrix on Diff-Quik staining (4 cases). Less common background features were clean (2 cases), bloody (2 cases), serous (1 case), and cystic (1 case). Necrotic background was noted in 1 case (Figs 3-5).

The MSRSGC diagnostic category was *malignant* in the majority of FNA specimens (19 of 38; 50%). Ten of 38 cases (26%) were categorized as *suspicious for*

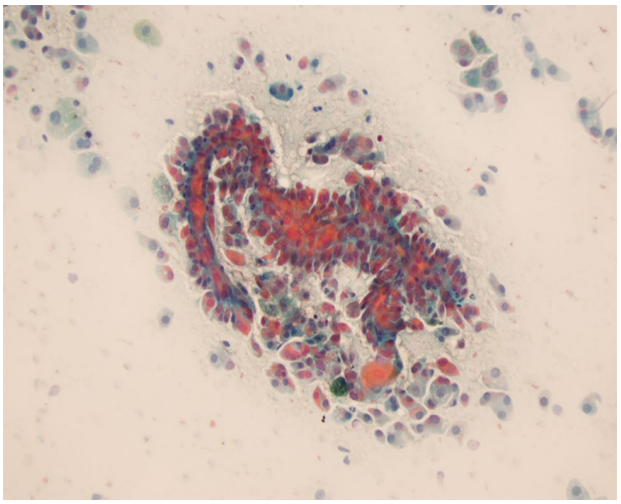

Figure 4. Neoplastic cells are arranged as papillary structures with fine fibrovascular cores, small clusters, and single cells (original magnification ×200, Papanicolaou stain).

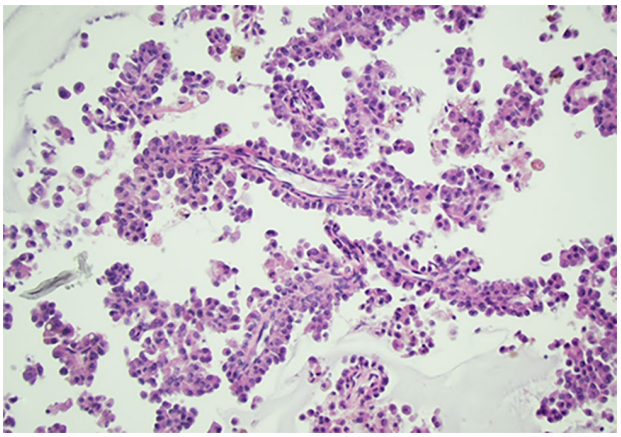

Figure 5. Numerous papillary structures with fine fibrovascular cores are seen in a cell block preparation (original magnification ×100, H&E stain).

malignancy, and 7 of 38 (18%) were classified as *salivary gland neoplasm of uncertain malignant potential*. Two of 38 cases (6%) were categorized as *atypia of undetermined significance*. Biopsies of metastatic sites (2 of 40 cases; 5%) were excluded from MSRSGC categorization, although both were diagnosed as *malignant*. No FNA cases were classified as *benign neoplasm* or *nonneoplastic*. The key cytomorphologic findings and the MSRSGC classification of the SC cases are summarized in Table 2.

Histochemical and immunohistochemical staining was used on FNA and resection specimens to further characterize the neoplastic cells (Table 3). The neoplastic cells were immunoreactive for S100 (18 of 22 cases), mammaglobin (18 of 18 cases) (Fig. 6), GATA-3 (8 of 8 cases), AE1/AE3 (7 of 7 cases), and vimentin (5 of 5 cases). Myoepithelial markers, including SMA (0 of 5 cases), calponin (0 of 5 cases), and P63 (0 of 12 cases), were negative. The Ki67 proliferation index was low in 5 of 6 cases and was from 30% to 40% in 1 of 6 tested cases. DOG-1 was negative in all 5 cases. GCDFP-15, Her2Neu, and estrogen receptor were negative (n = 2 each). Androgen receptor was negative in 2 cases (0 of 2 cases). A range of additional immunostains was used based on cytomorphologic features and clinical suspicion for secondary neoplasms, including 34Be12 (2 of 2 cases), BRST-2 (0 of 2 cases), CD10 (0 of 1 case), cytokeratin 7 (7 of 7 cases), epithelial membrane antigen (3 of 3 cases), glial fibrillary acidic protein (0 of 2 cases), thyroid transcription factor 1 (0 of 6 cases), carcinoembryonic antigen (0 of 2 cases), PAX8 (0 of 2 cases), SOX10 (3 of 4 cases), CD68 (1 of 1 case), TGB (0 of 1 case), calcitonin (0 of 1 case), Napsin A (0 of 1 case), WT-1 (0 of 1 case), cancer antigen 125 (0 of 1 case), CDX2 (0 of 1 case), and cytokeratin 20 (0 of 1 case).

Immunostaining and histochemical staining were performed on 28 of 40 resected SC specimens to confirm the diagnosis. The SC cases were positive for S100 (23 of 24 cases), GATA3 (13 of 13 cases), mammaglobin (17 of 17 cases), SOX10 (3 of 3 cases), and vimentin (6 of 6 cases) and were negative for DOG-1 (0 of 7 cases) and androgen receptor (0 of 3 cases). Similar to cytology cases, a wide range of immunostains was performed on resected SCs to exclude other malignancies or other primary sites, such as thyroid transcription factor 1 (0 of 5 cases), and PAX8 (0 of 2 cases). Histochemical studies indicated that tumor cells were positive for periodic acid-Schiff-diastase in 4 of 6 cases and for mucicarmine in 3 of 7 cases.

Molecular testing was performed to detect *ETV6-NTRK3* fusion in 33 of 40 cases (82.5%), 32 by FISH (32 of 33 cases) and 1 by next-generation sequencing (ThyroSeq). *ETV6* rearrangement was confirmed in 32 of 33 cases (96.7%). The tumor with no detectable fusion (1 of 33 cases; 3%) was from a man aged 47 years with a confirmed history of SC of the cheek who presented with a supraclavicular metastasis on which the negative FISH study was performed on FNA material. Molecular genetic testing results for *ETV6* rearrangement are summarized in Table 3.

TABLE 2. Cytomorphologic Features of Secretory Carcinoma Fine-Needle Aspiration Cases and Milan System for Reporting Salivary Gland Cytology Category

	Cytomorphology (No. of Cases)					
Sample Type	Cellularity	Architecture	Cell Morphology	Nucleus	Cytoplasm	Background
Smears (n = 32)	High cellularity (27) Crowding (27)	Sheets and clusters (27) Micropapillae and thin papillae (13) Single cells (12) Transgressing vessels (2) Acinar formation (2) Tubular formation (1) Stripped nuclei (2) Fibrovascular cores (1)	Large-to-medium size (28) Round-to polygonal (28) Epithelioid (5) Clear cell (2) Minimal atypia (6) Cyst-lining type (1) Small duct-type cells (1) Short spindle cell (1) Hobnail (1)	Round-to-oval (32) Eccentric (18) Central (14) Smooth contour (3) Small nucleoli (2) Large nucleoli (20) Fine-to-coarse chromatin (2)	Moderate-to-abundant (29) Vacuolated including large and small vacuoles (23) Finely granular (11) Clear (2)	Abundant mucin (8) Bubbly secretions (2) Granular with amorphous proteinaceous debris (9) Clean (2) Cystic (1) Serous fluid (1) Necrotic (1) Bloody (2) Multinucleate giant cells (4) Macrophages, including hemosiderin-laden macrophages (5) Lymphocytes (10) Neutrophils (3) Metachromatic matrix, fibrillary or spherical (3)
Cell blocks	Same	Same	Oncocytic (5) Ductal (5) Plasmacytoid (1)	Rare mitoses (7) Inclusions (2) Binucleation (2)	Enhanced clearing/eosinophilia (1)	Same
Milan system diagnostic category for FNA diagnosis						
Atypia of undetermined significance	6.0% (2)					
Salivary gland neoplasm of uncertain malignant potential	18.0% (7)					
Suspicious for malignancy	26.0% (10)					
Malignant	50.0% (19)					
Not applicable, metastatic site	5.0% (2)[a]					

Abbreviation: FNA, fine-needle aspiration.
[a]Both cases were diagnosed as malignant.

TABLE 3. Ancillary Histochemical/Immunohistochemical and Molecular Studies Performed on Fine-Needle Aspiration Cases of Secretory Carcinoma

			Variable Expression	
Ancillary Studies	Positive IHC	Negative IHC	Positive	Negative
FNA histochemistry/IHC (no./total no.)	Mammaglobin (18/18) GATA-3 (8/8) Vimentin (5/5) CK7 (7/7) AE1/AE3 (7/7) EMA (3/3) 34BE12 (2/2) CK19 (1/1)	DOG-1 (0/5) P63 (0/12) SMA (0/5) Calponin (0/5) TTF-1 (0/6) β catenin (0/2) ER (0/2) GCDFP-15 (0/2) Her2Neu (0/2) Pax8 (0/2) AR (0/2) CEA (0/2) Calcitonin (0/1)	S100 (18/22) SOX10 (3/4) Mucicarmine (1/4) PASD (2/3)	S100 (4/22) SOX10 (1/4) Mucicarmine (3/4) PASD (1/3)
Resection histochemistry/IHC (no./total no.)	Mammaglobin (17/17) GATA-3 (13/13) Vimentin (6/6) CK7 (7/7) SOX10 (3/3) BRST-2 (2/2) GCDFP-15 (2/2) EMA (1/1) AE1/AE3 (1/1) CKHMW (1/1) CK5/6 (1/1) BerEP4 (1/1)	DOG-1 (0/7) AR (0/3) Pax8 (0/2) Thyroglobulin (0/2) ER (0/1) HMB45 (0/1) β catenin (0/1) TTF-1 (0/5) P40 (0/1) P16 (0/1) SMA (0/1)	S100 (23/24) Calponin (1/3) PASD (4/6) Mucicarmine (3/7) P63 (2/9)	S100 (1/24) Calponin (2/3) PASD (2/6) Mucicarmine (4/7) P63 (7/9)
Molecular profile (no.) *ETV6-NTRK3, t(12;15)(p13;q25)*	Detected with FISH: 96.7% (32) Not detected with ThyroSeq NGS: 2.3% (1) Not performed: 17.5% (7)			

Abbreviations: AR, androgen receptor; CEA, carcinoembryonic antigen; CK, cytokeratin; EMA, epithelial membrane antigen; ER, estrogen receptor; FISH, fluorescence in situ hybridization; FNA, fine-needle aspiration; IHC, immunohistochemistry; PASD, periodic acid-Schiff-diastase; TTF-1 thyroid transcription factor 1.

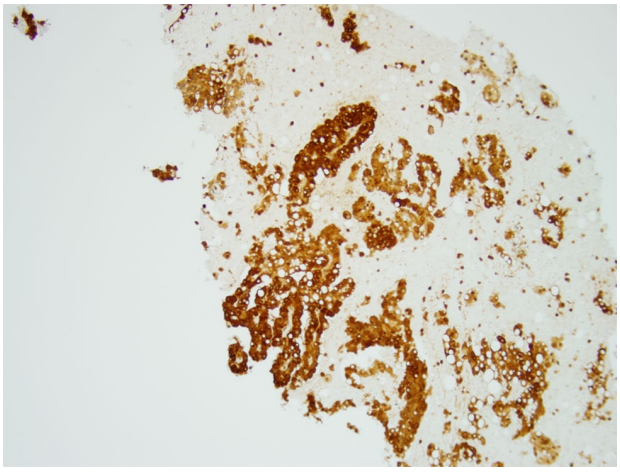

Figure 6. Mammaglobin immunostain highlights neoplastic cells in secretory carcinoma in a cell block preparation (original magnification ×100, immunostain).

Metastases were identified in 11 of 40 cases (27.5%). Of these, 9 of 11 cases exhibited spread to regional cervical lymph nodes, with 2 of 9 cases showing extranodal extension and 2 of 9 cases showing distant metastasis to brain and lungs in addition to regional lymph node spread. Two of 40 cases demonstrated only distant metastasis: 1 patient with metastases to the lungs and mediastinum and the other with metastasis to the liver.

Disease-related death occurred in 2 of 40 patients with lung metastasis. The time interval between tumor-related death and initial presentation was 13 months and 12 years, respectively.

DISCUSSION

The diagnosis of salivary gland SC in FNA specimens can be challenging. Its low-grade nature may make it difficult

to recognize from normal salivary gland elements or benign and malignant salivary gland neoplasms.

In 1 study, 109 cytologists were asked to classify a minor salivary gland case of SC. The diagnoses ranged from benign to malignant, and only 2 respondents were able to correctly identify SC based on morphologic features alone.[8]

The current study demonstrates that cellular architecture, cytomorphologic features and the background findings together are helpful in diagnosing SC. Cellular smears consisting of tight and loosely cohesive clusters, sheets, large and small 3-dimensional papillary groups, numerous single cells, naked nuclei, and transgressing vessels are common cellular arrangements. The neoplastic cells are large-to-medium in size and round-to-polygonal, with moderate-to-abundant cytoplasm. The cytoplasm mainly contains vacuoles (large vacuoles are more common than small vacuoles). Fine cytoplasmic granules and clear cell features are other cytoplasmic findings. The nuclei are round-to-oval, with smooth contour and minimal irregularity, and are eccentrically or centrally located. The nuclei consist of 1 or 2 nucleoli. Rare mitoses were seen. In the study mentioned above, the architectural atypia was considered more overt than any cellular or nuclear atypia.[8]

One cytologic hallmark of malignancy observed in the current study that is highlighted only rarely in published reports of SC is numerous dispersed tumor cells accompanying sheets and clusters.[5] In the absence of conventional high-grade features, including necrosis, mitoses, and prominent pleomorphism, the presence of single cells and crowded sheets and clusters is most indicative of a low-grade malignancy, especially when correlated with radiologic studies affirming a mass lesion.[9]

The background of FNA smears of SC can exhibit various contents, ranging from bloody background to abundant mucin, cyst fluid, and granular amorphous debris along with multinucleate giant cells, hemosiderin-laden macrophages, neutrophils, and lymphocytes. Only 1 of 40 cases of SC contained necrotic material in the background: The aspirated material was collected from a patient who died later from SC.

Metachromatic, fibrillary, or spherical-shaped matrix material was observed rarely in SC cases in this cohort. SC can rarely display an adenoid cystic carcinoma-like tubular and cribriform architecture on resection.[10] An SC case revealed tubular-like architecture and a metachromatic fibrillary matrix on Diff-Quik staining and lymphocytes in the background on aspirated material in the current study. The differential diagnosis included mucoepidermoid carcinoma and adenoid cystic carcinoma.

Cystic changes seen in radiologic studies as cystic background contents or as a cytomorphologic feature could be troublesome for diagnosing SC.[11] Cystic changes yielding limited cellular material, confoundingly, could raise the possibility of other neoplasms, such as low-grade mucoepidermoid carcinoma. However, such cases may fall under the category atypia of undetermined significance, prompting appropriate subsequent clinical follow-up, such as repeat biopsy. Other cystic cases may be definitively identified by molecular testing if sufficient cellularity is present.[12]

High-grade features were reliably absent from FNA smears of SC, although high-grade transformation of SC is known as a rare phenomenon.[13,14] Mitotic activity was reported to be prevalent in SC in 1 study; however, only rare mitotic activities were observed in our study.[15] SC may pose diagnostic dilemmas when it comes to tumor grading. In a blinded study conducted by a group of experts in salivary gland cytology, a case of SC had the highest rate of indeterminate responses (42.1%), indicating the diagnostic challenges based on cytomorphology alone.[16] Although tumor grading can be done with a high degree of accuracy based on cytomorphology alone in most instances, SC is labeled as *not gradable* or *indeterminate* in challenging cases.[16] This can contribute to a subset categorized as salivary gland neoplasm of uncertain malignant potential in the MSRSGC.[12,17,18] A mucinous background in SC and the presence of large cells with intracytoplasmic vacuoles can resemble mucoepidermoid carcinoma. Also, the large, round-to-polygonal cells with intracytoplasmic vacuoles or granules in SC can be mistaken for AciCC. Naked nuclei and the presence of lymphocytes in the background are additional cytomorphologic features overlapping with AciCC.[12] The oncocytic cells of SC with abundant cytoplasm and bland-appearing nuclei that have a smooth nuclear contour in a background of amorphous proteinaceous debris and tumor-associated lymphoid proliferation can mimic Warthin tumor.[17,19] SC may share cytomorphologic features with mucoepidermoid carcinoma or AciCC.[12] Malignant melanoma was considered in the differential diagnosis as a

metastatic process. In addition, oncocytoma (oncocytic features), chronic sialadenitis (oncocytic features, presence of matrix, and inflammatory cells), pleomorphic adenoma (large vacuolated cells, mucoid matrix, and inflammatory cells in the background), and Warthin tumor (oncocytic cells in the background of lymphocytes and mucoid material) were listed in the differential diagnosis in the current study.

Within the MSRSGC, only 2 cases were classified as atypia of undetermined significance, and 1 exhibited atypical epithelioid cells. The cell blocks in both cases were insufficient for ancillary studies. A recent study demonstrated that ancillary studies performed on cell blocks prepared from aspirated salivary gland material could enhance diagnostic yield.[20]

In the current study, the application of ancillary studies on material obtained by FNA of salivary glands assisted in reaching a definitive diagnosis of malignancies in 19 of 38 SC cases (50%) and of suspicious for malignancy in 10 of 38 SC cases (26%). This cohort of SC FNA cases showed an immunoprofile with positive staining for mammaglobin, GATA3, and S-100 and negative staining for DOG-1 and most myoepithelial markers. Although 2 biopsy cases demonstrated negative S100 staining, this may have been secondary to intratumoral heterogeneity. It has been proposed that accurate the diagnosis of SC is possible in the context of appropriate cytomorphologic features and a supporting immunophenotype of S-100 and mammaglobin positivity.[21-25] Distinguishing SC from AciCC by immunohistochemistry can be performed with DOG-1 and NR4A3, both of which are positive in AciCC. Recently, MUC4 has been found to be both sufficiently sensitive and sufficiently specific for SC to aid in its distinction from normal salivary gland tissue, ductal carcinomas of the salivary gland, and AciCC.[26,27] MUC4 has been shown to have 90% sensitivity and 100% specificity in distinguishing SC from mimics on histologic sections.[26] In addition, pan-TRK staining reportedly has near 100% specificity for SC in resection specimens.[28,29] Pan-TRK immunostains are reliable for identifying SC cases bearing an *ETV6-NTRK3* fusion, but these are not entirely specific for tumors with variant fusion genes.[29,30] Like in other salivary gland neoplasms, cytokeratin 7 and SOX10 are expressed in SC, whereas p63 and other myoepithelial markers are usually negative.[15,31,32]

AciCC is the most important differential diagnosis characterized by positive staining with DOG-1 and negative staining with mammaglobin. A recent study demonstrated high sensitivity and specificity of NR4A3 immunostaining for AciCC in cytology and surgical specimens.[33] Histochemical stains can be nonspecific in SC.[34] Periodic acid-Schiff-diastase positivity is observed in large cytoplasmic granules, leading to confusion with AciCC. SC with both macrocystic and microcystic features can show more extensive mucicarmine positivity, leading to confusion with mucoepidermoid carcinoma.[26,29,30]

An *ETV6-NTRK3* fusion gene, the genetic hallmark of SC, was detected in 32 of 33 cases in which genetic testing was performed, confirming the diagnosis. Rarely, SC shows lack of an *ETV6-NTRK3* fusion, like 1 of 33 cases in this study. Since its initial description, SC is now known to harbor variant fusions with *ETV*, including *RET*, *MET*, and *MAML3*.[13,14,35] *ETV6-RET* fusion is probably the second most common fusion gene in SC after *ETV6-NTRK3*.[13,14] Cases of SC with high-grade morphology and aggressive behavior have been identified with *ETV6-MET* fusions or simultaneous *ETV6-NTRK3* and *MYB-SMR3B* fusions. Molecular profiling of SC is of paramount importance in patients with advanced-stage SC harboring *NTRK* fusions because they may benefit from *TRK*-inhibitor therapy.[36] The identification of *ETV6* rearrangement still remains diagnostic in most cases of SC.

Interestingly, a small subset of SCs lack *ETV6* rearrangement, including rare cases with *CTNNA1-ALK* fusion and another (a *VIM-RET* fusion), as well as cases with no documented fusion gene.[13,37] Molecular testing may be valuable in confirming the diagnosis of SC arising in unusual locations, including thyroid, vulva, lung, and lacrimal gland.[36,38-41] These variable molecular changes associated with SC re-emphasize the importance of interpreting cytomorphology alongside molecular findings. Some tumors may not be obliging to demonstrate a hallmark genetic finding.

This study was retrospective and thus limited the statistical analyses. Central review was not performed because of the distant geography of participant institutions. In addition, molecular tests were not available for all cases.

In conclusion, SC of the salivary gland is a rare entity that may pose diagnostic challenges. Awareness of its

cytologic features is paramount to achieve an accurate diagnosis. Together, the architecture, cytomorphologic features, and the background content of SC, along with a general lack of high-grade features, can be suggestive of SC. These characteristics allow for both recognition of a neoplasm and subsequent ancillary testing. Morphologic interpretation must be supported by an immunohistochemical profile and molecular studies to confirm a diagnosis of SC.

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AUTHOR CONTRIBUTIONS

Austin B. Wiles: Writing–original draft, data curation, formal analysis, investigation, methodology, resources, software, validation, and writing–review and editing. **Matthew Gabrielson:** Data curation, investigation, resources, and writing–review and editing. **Zubair W. Baloch:** Data curation, investigation, resources, and writing–review and editing. **William C. Faquin:** Data curation, investigation, resources, and writing–review and editing. **Vickie Y. Jo:** Data curation, investigation, resources, and writing–review and editing. **Fabiano Callegari:** Data curation, investigation, resources, and writing–review and editing. **Ivana Kholova:** Data curation, investigation, resources, and writing–review and editing. **Sharon Song:** Data curation, investigation, resources, and writing–review and editing. **Barbara A. Centeno:** Data curation, investigation, resources, and writing–review and editing. **Syed Z. Ali:** Data curation, investigation, resources, and writing–review and editing. **Satu Tommola:** Data curation, investigation, resources, and writing–review and editing. **Guido Fadda:** Data curation, investigation, resources, and writing–review and editing. **Gianluigi Petrone:** Data curation, investigation, resources, and writing–review and editing. **He Wang:** Data curation, investigation, resources, and writing–review and editing. **Esther D. Rossi:** Data curation, investigation, resources, and writing–review and editing. **Liron Pantanowitz:** Data curation, investigation, resources, and writing–review and editing. **Zahra Maleki:** Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing–original draft, and writing–review and editing.

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