Application of the Milan System for Reporting Salivary Gland Cytopathology to Cystic Salivary Gland Lesions

Zahra Maleki, MD [1]; Derek B. Allison, MD [2]; Monica Butcher, MD[1]; Satomi Kawamoto, MD[3]; David W. Eisele, MD[4]; and Liron Pantanowitz, MD [5]

BACKGROUND: Cystic salivary gland lesions present diagnostic challenges on fine-needle aspiration (FNA) specimens that are related to sampling limitations and a broad differential diagnosis. This study evaluated the benefit of applying the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) to a series of cystic salivary gland lesions. **METHODS:** The pathology archives at the Johns Hopkins Hospital were searched to identify cystic salivary gland FNA specimens over a 19-year period (2000-2018). Patient demographics, cytomorphologic features, and clinical and surgical follow-up were recorded. The MSRSGC was applied to the cases. The risk of malignancy (ROM) and the risk of neoplasia (RON) were calculated for each category. **RESULTS:** One hundred seventy-eight cases were identified (96 males and 82 females) with a mean age of 53 years (range, 4-90 years). After the MSRSGC was applied, there were 52 nondiagnostic cases (29.2%), 80 nonneoplastic cases (44.9%), 35 cases of atypia of undetermined significance (AUS; 19.7%), 3 benign neoplasms (1.7%), 3 salivary gland neoplasms of uncertain malignant potential (SUMP; 1.7%), 4 cases suspicious for malignancy (SFM; 2.2%), and 1 malignant case (0.6%). One hundred fifty-six of the 178 patients (87.6%) had follow-up data available. The RON and ROM values for cases with surgical follow-up were 33.3% (3 of 9) and 22.2% (2 of 9) for the nondiagnostic category, 42.9% (9 of 21) and 19% (4 of 21) for the nonneoplastic category, 76.5% (13 of 17) and 29.4% (5 of 17) for the AUS category, 100.0% (2 of 2) and 50.0% (1 of 2) for the SUMP category, and 100% (2 of 2) and 100% (2 of 2) for the SFM category, respectively. **CONCLUSIONS:** Applying the MSRSGC to cystic salivary gland lesions improves patient management by preventing unnecessary surgery for nonneoplastic conditions. The ROM was highest in the SFM category (100%), which was followed by the SUMP, AUS, nondiagnostic, and nonneoplastic categories. Less than adequate specimens may increase the diagnosis of AUS. ***Cancer Cytopathol* 2021;129:214-225**. © *2020 American Cancer Society*.

KEY WORDS: cyst; cystic salivary gland lesions; cytology; cytopathology; fine-needle aspiration (FNA); head and neck pathology; Milan System for Reporting Salivary Gland Cytopathology; salivary gland.

INTRODUCTION

Fine-needle aspiration (FNA) cytology is a well-accepted procedure for the preoperative evaluation of salivary gland mass lesions.[1] Salivary gland FNA is minimally invasive and cost-effective with high accuracy for discriminating between nonneoplastic and neoplastic entities.[1,2] It is highly specific for the detection of neoplasia (98%) and malignancy (96%). However, it is less sensitive for the detection of neoplasia (up to 96%) and malignancy (up to 79%).[3] Salivary gland FNA is a valuable clinical decision-making tool that can prevent potentially unnecessary surgical resection in patients diagnosed with nonneoplastic conditions.[4]

Corresponding Author: Zahra Maleki, MD, Division of Cytopathology, Department of Pathology, Johns Hopkins Hospital, 600 N Wolfe St, Pathology 412C, Baltimore, MD 21287 (zmaleki1@jhmi.edu).

[1]Department of Pathology, Johns Hopkins Hospital, Baltimore, Maryland; [2]Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington, Kentucky; [3]Department of Radiology, Johns Hopkins Hospital, Baltimore, Maryland; [4]Department of Otolaryngology–Head and Neck Surgery, Johns Hopkins Hospital, Baltimore, Maryland; [5]Department of Pathology, University of Michigan, Ann Arbor, Michigan

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Salivary gland neoplasms are uncommon and account for only 6% of all head and neck neoplasms and 0.3% of all malignancies in the United States.[5] Cystic salivary gland lesions are rare and account for up to 8% of all salivary gland lesions. However, a number of nonneoplastic and neoplastic processes may present as cystic lesions.[6] Salivary gland cytopathology may possess diagnostic difficulties due to tumor heterogeneity, metaplastic changes, and morphologic overlap between entities.[7] The interpretation of cystic salivary gland lesions is even more challenging because samples are hampered by low cellularity and often a nonspecific watery or mucoid background.[6,8] To address the specific challenges associated with salivary gland cystic lesions, an algorithmic approach based on the presence or absence of mucin, epithelium, and/or lymphocytes was suggested.[6,8] However, there previously was no universal system for reporting cytomorphologic findings for cystic salivary gland lesions or the associated risk of malignancy (ROM) and risk of neoplasia (RON). The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), published in 2018,[9] is a risk-stratification classification scheme in which the ROM has been defined for each diagnostic category. Although data exist on the general performance of salivary gland FNA, less is known specifically about cystic salivary gland lesions and their impact based on the MSRSGC. The diagnostic utility of preoperative FNA cytology for cystic major salivary gland lesions was evaluated in our previous study,[10] but this was before the adoption of the MSRSGC. In this study, the application of the MSRSGC to cystic salivary gland lesions was investigated, and the ROM and the RON were calculated for each diagnostic category.

MATERIALS AND METHODS

The institutional review board of the Johns Hopkins Medical Institutions approved this study. Cases were identified through a search of the electronic pathology archives of the Johns Hopkins Hospital over a 19-year period (January 1, 2000, to December 31, 2018) for FNA samples from major salivary gland lesions. Samples were defined as cystic by preprocedural ultrasound examination by a radiologist or by pre- and postprocedural palpation by an otolaryngologist/head and neck surgeon. For each case, the patient's age, sex, race, use of ultrasound guidance, and clinical nonsurgical and surgical follow-up were recorded.

The MSRSGC was applied for each case as follows[10,11]:

- Nondiagnostic: specimens consisting of nonmucinous cystic fluid only.
- Nonneoplastic: specimens containing any benign-appearing acinar or ductal epithelial components, abundant inflammatory cells, and/or inflammatory cells with amylase crystalloids.
- Atypia of undetermined significance (AUS): specimens consisting of abundant mucin with or without rare epithelial cells or specimens with rare atypical cells.
- Benign neoplasm: Warthin tumor or cystic pleomorphic adenoma.
- Salivary gland neoplasm of uncertain malignant potential (SUMP): epithelial cells such as oncocytic or oncocytoid neoplasms with a cystic background for which the differential diagnosis includes a Warthin tumor or oncocytic cystadenoma.
- Suspicious for malignancy (SFM): Specimens with atypical cells suspicious for any malignancy such as atypical cells in a mucinous background suspicious for low-grade mucoepidermoid carcinoma.
- Malignant neoplasm: Malignant cells in a cystic background such as keratinizing squamous cell carcinoma.

The diagnostic performance between FNA and follow-up data was recorded. Because of the importance of specific cytomorphologic findings, all cases from each Milan diagnostic category were examined for the most prominent features, including the presence of lymphocytes, mucin, acellular background debris containing macrophages (cyst contents), squamous cells, oncocytes, and any atypical cells. The Milan diagnostic category and the aforementioned prominent cytomorphologic features were correlated.

Data Analysis

ROM and RON were calculated for each diagnostic Milan category as follows:

RON = Number of neoplastic cases/Total cases of interest

ROM = Number of malignant cases/Total cases of interest

RESULTS

Patient Demographics

During the study period of 2000-2018, 178 cases met the inclusion criteria. There were 96 males (54%) and

82 females (46%) with an average age of 53 years (range, 4-90 years). There were 92 patients (51.7%) who were White, 60 (33.7%) who were Black, 10 (5.6%) who were Asian, and 2 (1.1%) who were Hispanic; the race was unknown in 14 cases. FNA sites included the parotid gland (172 [96.6%]), the submandibular gland (4 [2.2%]), and the sublingual gland (1 [0.6%]) as well as 1 specimen labeled "lymph node, level II" (1 [0.6%]). Ultrasound guidance with onsite evaluation was used in 62 cases (34.8%; Table 1).

Follow-Up

Clinical follow-up data were available for 156 patients (87.6%). Eighty-seven of these patients (55.8%) were initially managed conservatively with routine clinical evaluation and watchful waiting, 20 (13%) underwent repeat FNA, and 46 (30%) underwent surgical excision (Fig. 1). Additional studies were performed for 4 cases (20%) with repeat FNA; these included core biopsies in 3 cases and flow cytometry in 1 case. The mean follow-up for the conservatively managed group was 10.5 years (range, 0.5-16.3 years), and 3 of these patients (3.4%) eventually underwent repeat FNA after a mean follow-up of 6.2 years (range, 2.0-12.6 years). In addition, 5 patients (25%) who had initially undergone repeat FNA underwent surgical excision after a mean time period of 4.3 years (range, 0.08-6.3 years).

Application of the MSRSGC

The MSRSGC was applied to all FNA cases for the purposes of this study. There were 52 nondiagnostic cases (29.2%), 80 nonneoplastic cases (44.9%), 35 AUS cases (19.7%), 3 benign neoplasm cases (1.7%), 3 SUMP cases (1.7%), 4 SFM cases (2.2%), and 1 malignant case (0.6%). Clinical follow-up was available for 156 of these 178 patients (87.6%; Fig. 2).

Surgical Follow-Up

A total of 51 of the 178 cases (28.7%) underwent surgical excision (Table 1). The majority of the cases were diagnosed as nonneoplastic on FNA (n = 21); this category was followed by the AUS (n = 17), nondiagnostic (n = 9), SUMP (n = 2), and SFM categories (n = 2). Fourteen of the 51 cases (27.5%) were diagnosed as malignant on surgical follow-up; they included 6 of the 30 FNA cases with nonneoplastic diagnoses (20%), 5 of the 17 FNA cases diagnosed as AUS (29.4%), 1 of the 2 cases diagnosed as SUMP (50%), and 2 of the 2 cases diagnosed as SFM (100%). Malignant diagnoses on surgical follow-up are shown for each category in Table 1. Fifteen of the 51 cases (29.4%) were diagnosed as benign neoplasms on surgical follow-up, and they are listed for each category in Table 1. Nineteen of the 51 cases (37.3%%) were diagnosed as benign cysts on surgical follow-up, and they are listed for each category in Table 1.

Subsequently, the prominent morphologic features composing the cystic component (lymphocytes, mucin, acellular background debris containing scattered macrophages, squamous cells, oncocytes, and atypical cells) were correlated with surgical follow-up. Twenty-one of the 59 cases containing numerous lymphocytes (35.6%) had surgical follow-up. The benign diagnoses (n = 16) included lymphoepithelial cysts (n = 6), Warthin tumors (n = 4), an apocrine hydrocystoma (n = 1), a branchial cleft cyst (n = 1), a ruptured cyst with squamous lining (n = 1), benign lymphoid and fibrous tissue (n = 1), a cystadenoma with prominent lymphoid stroma (n = 1), and parotid tissue with reactive lymphocytes and fibrosis (n = 1). The malignant cases (n = 5) consisted of a cystic mucoepidermoid carcinoma (n = 1), an acinic cell carcinoma (n = 1), an Epstein-Barr virus (EBV)–associated smooth muscle tumor (n = 1), an EBV-positive diffuse large B cell lymphoma (n = 1), and a low-grade extranodal marginal zone lymphoma (mucosa-associated lymphoid tissue lymphoma; n = 1). Seven of the 12 cases with a prominent mucin component (58.3%) had surgical follow-up; they included 5 malignant cases and 2 benign cases reported as follows: mucoepidermoid carcinoma (n = 5), oncocytic cystadenoma (n = 1), and benign parotid tissue (n = 1). Among the 74 cases with predominantly acellular background debris containing scattered macrophages, 12 (16.2%) had surgical follow-up; there were 2 malignancies and 1 benign neoplasm, and the remainder (n = 9) were nonneoplastic conditions. These diagnoses included a papillary cystic variant of acinic cell carcinoma (n = 1), a mucoepidermoid carcinoma (n = 1), a pleomorphic adenoma (n = 1), a branchial cleft cyst (n = 1), a retention cyst (n = 1), a benign cyst with oncocytic epithelium (n = 1), chronic sialadenitis with duct dilation and oncocytic change (n = 1), salivary duct cysts (n = 4), and a benign squamous-lined cyst (n = 1). Nine of the 22 cases with prominent squamous cells (40.9%%) had surgical follow-up, and they included squamous cell carcinomas (n = 2), a Warthin tumor (n = 1), a cystic

TABLE 1. Salivary Gland FNA Cases Categorized According to the MSRSGC and Correlated With Their Surgical Follow-Up

MSRSGC	Sex: Male/Female	Age, Range (Mean), y	FNA Site	Ultrasound-Guided FNA	Surgical Follow-Up	RON	ROM
Nondiagnostic (9/52)	5/4	4-85 (51.9)	Parotid (9)	Yes (2) No (7)	Salivary duct cyst (3) Retention cyst (1) Squamous-lined cyst (1) Cyst with oncocytic epithelium (1) Pleomorphic adenoma (1) Mucoepidermoid carcinoma (1) Acinic cell carcinoma (1)	33.3% (3/9)	22.2% (2/9)
Nonneoplastic (21/80)	13/8	4-87 (52.1)	Parotid (21)	Yes (8) No (13)	Salivary duct cyst (4) Epidermal inclusion cyst (1) Bronchial cleft cyst (2) Lymphoepithelial cyst (4) Chronic sialadenitis (1) Apocrine hydrocystoma (1) Warthin tumor (3) Sebaceous lymphadenoma (1) Malignant EBV-associated smooth muscle tumor (1) Squamous cell carcinoma (1) Lymphoma (2)	42.9% (9/21)	19% (4/21)
AUS (17/35)	11/6	10-84 (53.7)	Parotid (16) Lymph node, level IIB (1)	Yes (10) No (7)	Benign salivary gland tissue (1) Benign lymphoid tissue (1) Squamous-lined cyst (1) Fibrous walled cyst (1) Pleomorphic adenoma (1) Warthin tumor (2) Cystadenoma (2) Lymphadenoma (2) Hemangioma (1) Mucoepidermoid carcinoma (3) Squamous cell carcinoma (1) Acinic cell carcinoma (1)	76.5% (13/17)	29.4% (5/17)
SUMP (2/3)	1/1	63-66 (64.5)	Parotid (2)	No (2)	Warthin tumor (1) Mucoepidermoid carcinoma (1)	100% (2/2)	50% (1/2)
Suspicious for malignancy (2/4)	1/1	15-82 (48.5)	Parotid (2)	No (2)	Mucoepidermoid carcinoma (2)	100% (2/2)	100% (2/2)
Total (51)	31/20	4-87 (53)	Parotid (50) Lymph node (1)	Yes (20) No (31)	Nonneoplastic (22) Benign neoplasm (15) Malignant neoplasm (14)	56.9% (29/51)	66.7% (14/21)

Abbreviations: AUS, atypia of undetermined significance; EBV, Epstein-Barr virus; FNA, fine-needle aspiration; MSRSGC, Milan System for Reporting Salivary Gland Cytopathology; ROM, risk of malignancy; RON, risk of neoplasm; SUMP, salivary gland neoplasm of uncertain malignant potential.
The presented values are n values unless otherwise indicated. ROM and RON were calculated only for FNA cases with surgical follow-up.

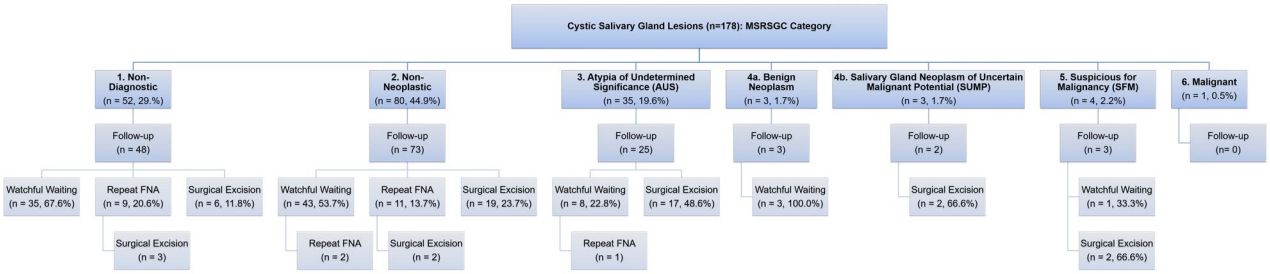

Figure 1. Diagram of the application of the MSRSGC to cystic salivary gland lesions and their follow-up. FNA indicates fine-needle aspiration; MSRSGC, Milan System for Reporting Salivary Gland Cytopathology.

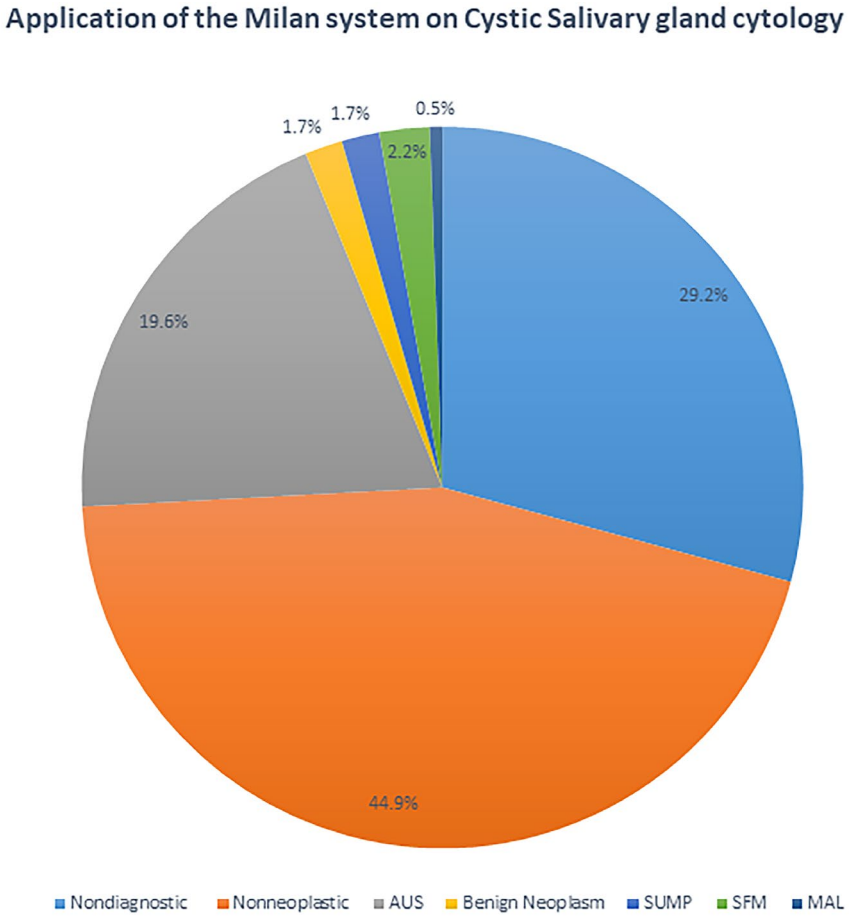

Figure 2. A pie chart shows the distribution of cases in each Milan system category. AUS indicates atypia of undetermined significance; MAL, malignancy; SFM, suspicious for malignancy; SUMP, salivary gland neoplasm of uncertain malignant potential.

sebaceous lymphadenoma (n = 1), salivary duct cysts (n = 3), an epidermal inclusion cyst (n = 1), and a fibrous walled cyst with keratin debris and reactive atypia (n = 1). Three cases with prominent oncocytes had conservative follow-up. Among 6 cases with atypical cells, 2 (33.3%) had surgical follow-up; they included a pleomorphic adenoma (n = 1) and a hemangioma (n = 1).

Milan Categories and Their Associated Prominent Cytomorphologic Findings

Nondiagnostic category

The nondiagnostic category included 52 cases. The majority of these cases (n = 47) showed acellular cystic fluid and scattered macrophages. Numerous lymphocytes

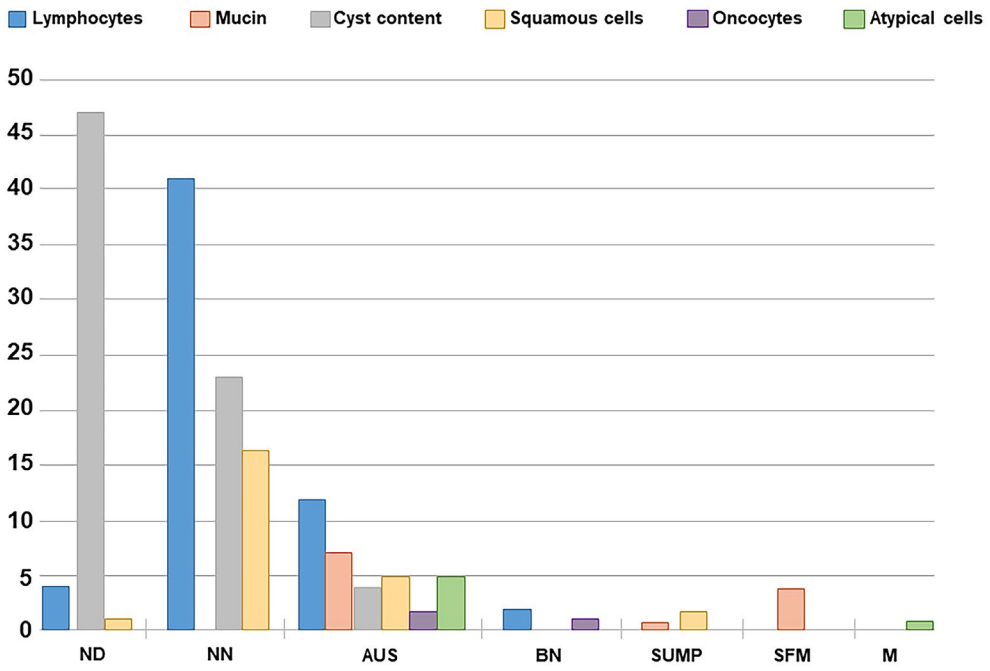

Figure 3. Distribution of the prominent cytomorphologic findings of each Milan system category. AUS indicates atypia of undetermined significance; BN, benign neoplasm; M, malignant; ND, nondiagnostic; NN, nonneoplastic; SFM, suspicious for malignancy; SUMP, salivary gland neoplasm of uncertain malignant potential.

were noted in 4 cases. Squamous cells were present in 1 case. Nine cases had surgical follow-up. A case with cyst contents containing numerous lymphocytes was a low-grade cystic mucoepidermoid carcinoma. One case with background cyst contents with associated benign-appearing acinar cells was diagnosed as an acinic cell carcinoma, papillary cystic variant, on excision. Another case with cyst contents was determined to be a pleomorphic adenoma with cystic change on histology. The remaining cases on surgical follow-up included salivary duct cysts (n = 3), a retention cyst (n = 1), and a cyst with oncocytic epithelium (n = 1). One case with prominent squamous cells was aspirated from a benign squamous cell–lined cyst on histology (Fig. 3).

Nonneoplastic category

The nonneoplastic category included 80 cases. For cases placed within the nonneoplastic category, the prominent cytomorphologic findings were lymphocytes (n = 41), acellular background debris containing scattered macrophages (n = 23), and squamous cells (n = 16). Twenty-one cases had surgical follow-up. The surgical follow-up for 12 cases with prominent lymphocytes was as follows: lymphoepithelial cyst (n = 4), branchial cleft cyst (n = 1), Warthin tumor (n = 3), EBV-associated smooth muscle tumor (n = 1), apocrine hydrocystoma (n = 1), and lymphoma (EBV-positive diffuse large B cell lymphoma [n = 1] and low-grade extranodal marginal zone lymphoma [n = 1]). The surgical follow-up of cases with prominent background acellular debris and scattered macrophages revealed chronic sialadenitis with duct dilation and squamous and oncocytic metaplasia (n = 1), salivary duct cysts (n = 1), and a branchial cleft cyst (n = 1). Six cases with prominent squamoid features had surgical follow-up; they included a squamous cell carcinoma (n = 1), an epidermal inclusion cyst (n = 1), a cystic sebaceous lymphadenoma (n = 1), and salivary duct cysts with squamous metaplasia (n = 3).

AUS category

There were 35 cases included in this category (Fig. 4). Predominant features that were identified included squamous cells (n = 5), mucin (n = 7), oncocytes (n = 2), lymphocytes (n = 12), atypical cells (n = 5), and background cyst contents (n = 4). Seven of the 12 cases in this group with prominent lymphocytes had surgical follow-up, which revealed the following diagnoses: Warthin tumor with focal squamous metaplasia (n = 1),

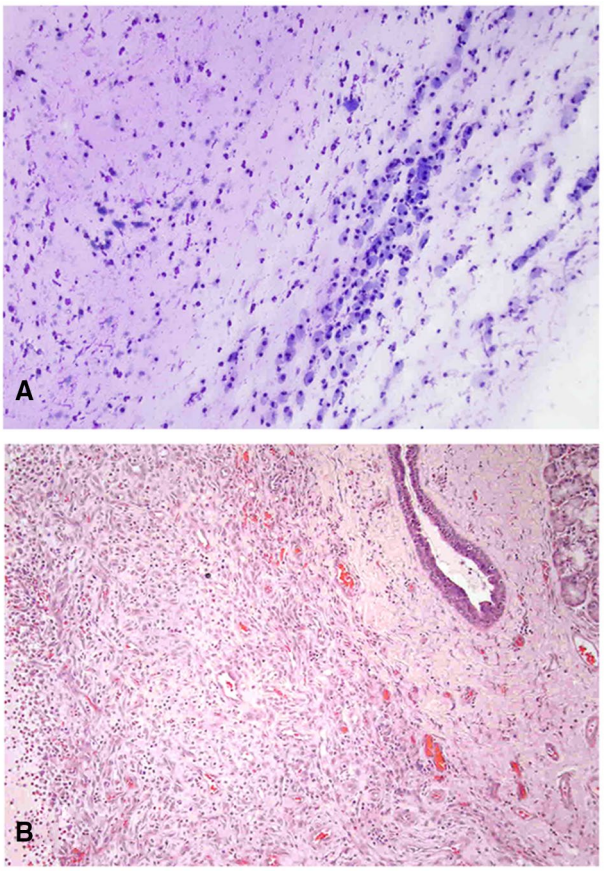

Figure 4. (A) Fine-needle aspiration of a 2-cm cystic submandibular mass showed scattered macrophages and lymphocytes in a background of mucin. This is an example of atypia of undetermined significance (Diff-Quik stain, ×200). (B) Surgical follow-up of the case showed a mucocele with granulation tissue and extravasated mucin (H & E, ×100).

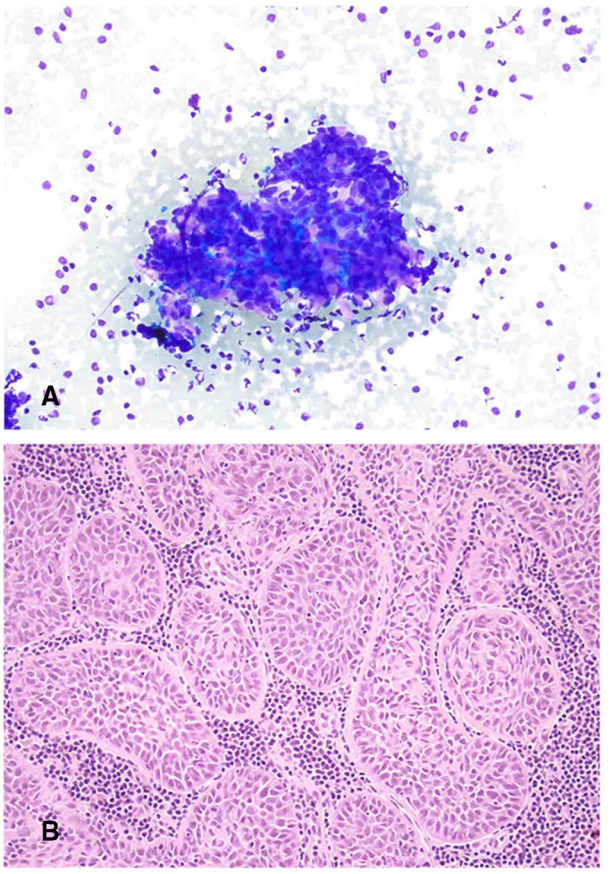

Figure 5. (A) Fine-needle aspiration of a 4-cm cystic parotid mass exhibited epithelial fragments with basaloid features and scant stroma; it was categorized as a salivary gland neoplasm of uncertain malignant potential (Papanicolaou stain, ×200). (B) Surgical follow-up led to a diagnosis of lymphadenoma (H & E, ×100).

acinic cell carcinoma (n = 1), lymphoepithelial cysts (n = 2), benign focally ruptured squamous-lined cyst (n = 1), cystadenoma with prominent lymphoid stroma (n = 1), and benign lymphoid tissue (n = 1). Four of the 7 cases with prominent mucin had surgical follow-up, and they were diagnosed as low-grade mucoepidermoid carcinomas (n = 2), an oncocytic cystadenoma (n = 1), and benign parotid tissue (n = 1). One of the 4 cases with cyst contents had surgical follow-up, and it was diagnosed as a mucoepidermoid carcinoma (n = 1). Two of the 5 cases with prominent squamous features had surgical follow-up; they included a case of squamous cell carcinoma (n = 1) and another case with a fibrous walled cyst containing keratin debris (n = 1). Two cases with oncocytes had no surgical follow-up. Three of the 5 cases with atypical cells had surgical follow-up, and they were diagnosed as a pleomorphic adenoma with florid metaplastic squamous differentiation and necrosis (n = 1), a Warthin tumor (n = 1), and a hemangioma (n = 1).

Benign neoplasm category

There were 3 benign neoplasms identified. Cytologic examination showed prominent lymphocytes in 2 of these cases consistent with a lymphoepithelial cyst (n = 2). The third case exhibited oncocytes and macrophages in a cystic background and was favored to be a Warthin tumor. All 3 of these cases were followed by watchful waiting.

SUMP category

Three cases were included in the SUMP category (Fig. 5). Two cases showed squamous epithelium, and 1 case contained mucin. Two of these 3 cases had surgical follow-up.

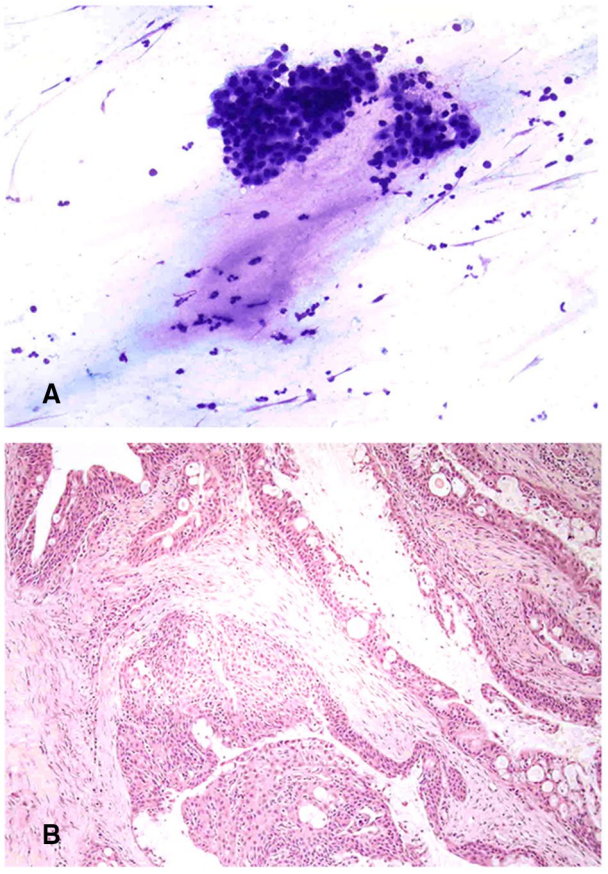

Figure 6. (A) Fine-needle aspiration of a 3-cm left parotid mass showed fragments of epithelial cells in a background of mucin suspicious for malignancy (Diff-Quik stain, ×200). (B) Surgical follow-up was consistent with an intermediate-grade mucoepidermoid carcinoma (H & E stain, ×100).

The case with mucin was diagnosed as a mucoepidermoid carcinoma on surgical follow-up. One case with squamous epithelium was attributed to a Warthin tumor on surgical follow-up.

SFM category

There were 4 cases included in this category (Fig. 6). All 4 cases contained mucin. In 2 of these cases, a retention cyst was favored on the basis of the cytologic and clinical findings. Two cases were diagnosed as mucoepidermoid carcinoma on surgical follow-up.

Malignant category

The 1 case in this category consisted of malignant epithelial cells present in a cystic background and was not followed at our institution.

Surgical Follow-Up of Cases Based on Their Prominent Cytomorphologic Findings

Surgical follow-up was available for 10 cases with prominent lymphocytes, and the diagnoses based on histopathology were as follows: lymphoepithelial cyst (n = 2), acinic cell carcinoma (n = 1), EBV-associated smooth muscle tumor (n = 1), EBV-positive diffuse large B cell lymphoma (n = 1), apocrine hydrocystadenoma (n = 1), branchial cleft cyst (n = 1), Warthin tumor with squamous metaplasia (n = 1), benign lymphoid tissue (n = 1), and cystadenoma with prominent lymphoid hyperplasia (n = 1). Seven cases with prominent mucin, including 5 mucoepidermoid carcinomas, 1 oncocytic cystadenoma, and 1 case with just benign parotid tissue, had surgical follow-up. Twelve cases with prominent acellular debris and few macrophages had surgical follow-up; they included an acinic cell carcinoma (n = 1), a mucoepidermoid carcinoma (n = 1), salivary duct cysts (n = 4), a pleomorphic adenoma (n = 1), a Warthin tumor (n = 1), a benign cyst with oncocytic epithelium (n = 1), chronic sialadenitis with duct dilation (n = 1), a branchial cleft cyst (n = 1), and a retention cyst (n = 1). Nine cases with prominent squamous features had surgical follow-up, and the follow-up diagnoses were squamous cell carcinoma (n = 2), salivary duct cyst (n = 3), cystic sebaceous lymphadenoma (n = 1), epidermal inclusion cyst (n = 1), fibrous walled cyst with keratin debris and reactive atypia (n = 1), and Warthin tumor (n = 1). Two cases with prominent atypical epithelial cells had surgical follow-up that showed a hemangioma (n = 1) and in another case a pleomorphic adenoma (n = 1).

RON and ROM for Each Category

RON and ROM were calculated for different settings: one for FNA cases with surgical follow-up and another for all FNA cases with any follow-up, including clinical and surgical follow-up. RON and ROM are tabulated for each category in Tables 1 and 2. RON in the nonneoplastic category for cases with surgical follow-up was 42.9% (9 of 21), and ROM was 19% (4 of 21). Interestingly, ROM in the nondiagnostic category with surgical follow-up was 22.2% (2 of 9), and this was higher than the value for the nonneoplastic category proposed by the MSRSGC. RON and ROM for the SFM category were both 100% (2 of 2). The overall RON value for all categories was 56.9% (29 of 51),

TABLE 2. Differences in RON and ROM Between Only Cases With Surgical Follow-Up and All Cases With Follow-Up

	Cases With Surgical Follow-Up Only		All Cases With Follow-Up	
MSRSGC	RON	ROM	RON	ROM
Nondiagnostic (n = 48)	33.3% (3/9)	22.2% (2/9)	6.25% (3/48)	4.2% (2/48)
Nonneoplastic (n = 73)	42.9% (9/21)	19% (4/21)	12.3% (9/73)	5.4% (4/73)
AUS (n = 25)	76.5% (13/17)	29.4% (5/17)	52% (13/25)	20% (5/25)
Benign neoplasm (n = 3)	NA	NA	100% (3/3)	0%
SUMP (n = 2)	100% (2/2)	50% (1/2)	100% (2/2)	50% (1/2)
Suspicious for malignancy (n = 3)	100% (2/2)	100% (2/2)	66.7% (2/3)	66.7% (2/3)
Total (n = 154)	56.9% (29/51)	27.5% (14/51)	18.8% (29/154)	9.1% (14/154)

Abbreviations: AUS, atypia of undetermined significance; MSRSGC, Milan System for Reporting Salivary Gland Cytopathology; NA, not applicable; ROM, risk of malignancy; RON, risk of neoplasia; SUMP, salivary gland neoplasm of uncertain malignant potential.

and ROM was 27.5% (14 of 51). The overall RON and ROM values were 18.8% (29 of 154) and 9.1% (14 of 154), respectively, for all FNA cases with follow-up, including clinical and surgical follow-up.

DISCUSSION

The clinical presentation for a wide variety of neoplastic and nonneoplastic salivary gland conditions may manifest as a cystic lesion.[10] Ultrasonography is often used to evaluate salivary cystic lesions. With ultrasound, simple cysts often appear as round or oval masses with well-delineated boarders, anechoic centers, and clear posterior acoustic enhancement.[12,13] Some cystic lesions are more complex on ultrasound and may contain variable degrees of heterogeneity with internal debris or septations. However, these radiologic features are nonspecific. Furthermore, overlapping features of benign and malignant cystic salivary gland conditions, the complexity of this anatomic site due to other potential non–salivary gland cystic structures, and the variable experience of the radiologist make the interpretation of these cysts on imaging even more challenging. In addition, lymph nodes with involvement by metastatic carcinoma, lymphoma, or even reactive changes can mimic salivary gland cysts.[14,15] As a result of these limitations of imaging, minimally invasive sampling of these lesions is paramount in driving clinical decision making and decreasing the number of patients ultimately undergoing unnecessary surgical interventions.[4,16] Before the MSRSGC, salivary gland cysts were evaluated solely on the basis of their cytomorphologic features, such as the presence of lymphocytes, mucin, or oncocytes.[6] The MSRSGC categorizes cysts into 1 of 6 tiers on the basis of their cytomorphologic findings. The nonneoplastic category in this study was the most commonly used category (n = 80 [44.9%]) with the highest number of surgical follow-up cases. None of the cytology cases in this series were categorized as benign. Repeat FNA was another modality used for follow-up in cases with nondiagnostic, nonneoplastic, and AUS diagnoses. Although the total number of cases with diagnoses of SUMP and SFM was very low, two-thirds of these cases underwent surgery. Over 28.6% of the cases (51 of 178) had surgical follow-up. The ROM values for the nondiagnostic, nonneoplastic, benign, atypical, SUMP, SFM, and malignant categories are 25%, 10%, 20%, <5%, 35%, 60%, and 90%, respectively, according to the MSRSGC.[9] In this study, the ROM values for the nondiagnostic, nonneoplastic, atypical, SUMP, and SFM categories were 22%, 19%, 29.4%, 50%, and 100%, respectively, for cystic salivary gland lesions; these values were higher than those for all salivary gland lesions. Interestingly, the ROM was slightly higher for the nondiagnostic category than the nonneoplastic category (22.2% [2 of 9] vs 19% [4 of 21]), but the difference was not statistically significant. The ROM for the nonneoplastic category was higher than the percentage proposed by the MSRSGC (19% [4 of 21] vs 10%); this can be explained by the small number of cases with surgical follow-up in this category. The overall RON and ROM values for cases with surgical follow-up were 56.9% (29 of 51) and 27.5% (14 of 51), respectively. One explanation for the higher ROM in cystic salivary gland lesions is that the majority of these lesions were treated conservatively with clinical watchful-waiting follow-up and occasionally repeat FNA, and only cases with a high clinical suspicion for malignancy underwent surgical intervention. The nondiagnostic category[17] and the nonneoplastic category constituted the majority of cases in the MSRSGC. Notably, the nondiagnostic

category is not equivalent to an insufficient specimen. Several studies have shown the significance of cyst components (mucin vs nonmucin) and also cellular features.[6,11] Cellular and noncellular components of all cystic lesions, including mucin, nonmucin cyst contents, lymphocytes, squamous cells, oncocytic cells, and atypical cells, were recorded in this study. Cyst contents were the main component in the majority of nondiagnostic cases, whereas lymphocytes were the main cellular finding in the nonneoplastic category. Mucin was present in a subset of atypical, SUMP, and SFM cases, and all 3 of these categories are more often associated with malignancy. Mucoepidermoid carcinoma can present as a hypocellular mucinous cyst, and this can lead to a misdiagnosis.[8] Because of these observations, an FNA specimen containing mucinous cyst fluid falls into the AUS category in the MSRSGC. A nonmucin cystic component was predominantly seen in nondiagnostic cases in this study, whereas cases with notable lymphocytes were predominantly seen among nonneoplastic cases. The atypical category constituted aspirates with the most variable cellular and acellular components.

The surgical follow-up diagnoses in this study confirmed prior observations showing that nonneoplastic and neoplastic lesions, including both benign and malignant entities, may clinically manifest as cysts.[8,18,19] According to surgical follow-up, nonneoplastic conditions included salivary duct cysts, mucus retention cysts, chronic sialadenitis, and lymphoepithelial cysts. According to surgical follow-up, benign neoplasms included pleomorphic adenoma, Warthin tumor, and lymphadenoma. According to surgical follow-up, malignant neoplasms included mucoepidermoid carcinoma, acinic cell carcinoma, and squamous cell carcinoma. Occasionally, branchial cleft cysts and lymphoepithelial cysts in human immunodeficiency virus–positive patients may be mistaken for cystic salivary gland lesions.[20,21] Lymphocytes can be seen in a wide variety of cystic salivary gland lesions. Lymphocytes are an essential element in rendering a diagnosis of benign conditions such as Warthin tumor and intraparotid lymph nodes. However, differentiating reactive lymphocytes from lymphomas is diagnostically challenging on the basis of cytomorphology alone without the use of ancillary studies. FNA of intraparotid lymph nodes is associated with a high false-positive rate (36%), and lymphoma is associated with a high false-negative rate (57%).[22] Furthermore, the radiologic findings of a reactive lymph node or a lymph node involved with metastatic carcinoma may overlap those of a cystic salivary gland.[14,15] Squamous cells are another morphologic finding in FNA samples that can contribute to diagnostic difficulties with cystic salivary glands. They can be seen in both nonneoplastic and neoplastic lesions, including benign and malignant neoplasms. Epidermal inclusion cysts, salivary duct cysts with squamous cell metaplasia, and squamous cell carcinomas can all present as cysts with variable degrees of squamous cell atypia.[7] Oncocytes are considered a typical cellular component of Warthin tumors and oncocytomas. However, oncocytes can also be seen in other nonneoplastic, metaplastic, and malignant conditions (eg, Warthin-like variant of mucoepidermoid carcinoma) presenting diagnostic challenges.[23,24] Our findings indicate that cystic salivary lesions may fall into different MSRSGC categories according to their cell type, cytomorphology, quality, and quantity. This does not suggest that the value of using the MSRSGC is limited in the setting of FNA of cystic salivary gland lesions. In fact, it echoes that the MSRSGC is similarly applicable to cystic salivary gland lesions as it is to solid salivary gland lesions. The surgical follow-up of several cases revealed entities that usually do not present as cysts, such as a pleomorphic adenoma, lymphomas, a malignant EBV-associated smooth muscle tumor, benign lymphoid tissue, and benign salivary gland tissue. The pleomorphic adenoma case showed degenerative changes and cyst formation with surgical follow-up. A mucosa-associated lymphoid tissue lymphoma case was associated with chronic sialadenitis and an adjacent 5.2-cm cyst. An EBV-positive diffuse large B cell lymphoma, a malignant EBV-associated smooth muscle tumor that contained an aggregate of lymphocytes on histology, and benign lymphoid tissue were all interpreted as lymphocytes suggestive of a lymphoepithelial cyst. Benign salivary gland tissue with an associated lymph node and no cyst was reported on surgical follow-up in the FNA of a 0.9-cm cystic lesion containing mucin. Benign salivary gland tissue with a fibrous walled cyst containing keratin debris and reactive changes was found on follow-up for a case with atypical squamous cells in a cystic background.

This study is limited by several factors. It is a retrospective study from a single institution and includes only 178 cases. However, salivary gland lesions are uncommon, and cystic salivary gland lesion are rare. A subset of cystic lesions may be followed only by watchful waiting without any interventions according to their clinical presentation. Therefore, those cystic lesions with a high suspicion

for a neoplastic process or malignancy are best evaluated by FNA. There were a few cases in the SUMP and SFM categories in this study, but only a fraction of them had surgical follow-up. Although the numbers for SUMP and SFM cases in this study might not be statistically significant, they are informative and can be used in future studies such as meta-analyses. The ROM and RON values of all categories might be affected by the fact that there were relatively few cases in each category. However, this study clearly shows that the application of the MSRSGC to cystic salivary gland lesions guided the clinicians toward a more conservative approach and prevented unnecessary surgeries in a substantial number of cases.

In conclusion, FNA is a minimally invasive and cost-effective diagnostic tool for helping to evaluate cystic salivary gland lesions[4,16] with a high diagnostic accuracy rate.[10,25] FNA of cystic salivary gland lesions provides valuable information about the nature of the lesions, which may prevent unnecessary surgeries in a considerable number of cases, as demonstrated in this study. Application of the MSRSGC shows that the majority of cystic salivary gland cases fall into the nondiagnostic category, which is followed by nonneoplastic aspirates. The ROM for cystic salivary gland lesions of all categories with surgical follow-up is slightly higher than that proposed by the MSRSGC, and this is likely attributable to the fact that only cases with a high suspicion for malignancy undergo surgical intervention. However, the RON and ROM values fall within the MSRSGC-proposed range when they are calculated for all FNA cases with any follow-up, including clinical and surgical follow-up.

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AUTHOR CONTRIBUTIONS

Zahra Maleki: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing–original draft, and writing–review and editing. **Derek B. Allison:** Conceptualization, data curation, formal analysis, investigation, methodology, validation, and writing–review and editing. **Monica Butcher:** Formal analysis, investigation, methodology, validation, and writing–review and editing. **Satomi Kawamoto:** Formal analysis, investigation, methodology, validation, and writing–review and editing. **David W. Eisele:** Formal analysis, investigation, methodology, validation, and writing–review and editing. **Liron Pantanowitz:** Conceptualization, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing–original draft, and writing–review and editing.

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