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

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The application of the Milan System for Reporting Salivary Gland Cytopathology to cystic salivary gland lesions provides valuable information in guiding clinical management, which may prevent unnecessary surgery in a considerable number of cases. The RON and ROM were 6.25%/4.2% for non-diagnostic, 12.3%/5.4% for non-neoplastic, 52%/20% for atypia of uncertain significance (AUS), 100%/0% for benign neoplasm, 100.0%/50.0% for salivary gland neoplasm of uncertain malignant potential (SUMP), and 66.6%/66.6% for the suspicious for malignancy (SFM) category, respectively.

Social Media & Promotion

The application of the Milan System for Reporting Salivary Gland Cytopathology to cystic salivary gland lesions provides valuable information in guiding clinical management, which may prevent unnecessary surgery in a considerable number of cases.

Key words: The Milan System for Reporting Salivary Gland Cytopathology, salivary gland, cyst, cystic salivary gland lesions, fine needle aspiration (FNA), cytopathology, cytology, head and neck pathology

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Abstract

Background: Cystic salivary gland lesions present diagnostic challenges on fine needle aspiration (FNA) specimens which is related to sampling limitations/ broad differential diagnosis. Herein, we evaluate the benefit of applying The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) to a series of cystic salivary gland lesions.

Design: The pathology archives at The Johns Hopkins Hospital was searched to identify cystic salivary gland FNAs over an 18 year period (2000 – 2018). Patient demographics, the cytomorphologic features, clinical and surgical follow-up were recorded. The MSRSGC was applied to cases. The risk of malignancy (ROM) and risk of neoplasia (RON) for each category were calculated.

Results: 178 cases were identified (96 males and 82 females) with a mean age of 53 years (4 – 90 years). After applying the MSRSGC, there were 52 (29.2%) non-diagnostic, 80 (44.9%) non-neoplastic, 35 (19.6%) atypia of undetermined significance (AUS), 3 (1.7%) benign neoplasms, 3 (1.7%) salivary gland neoplasms of uncertain malignant potential (SUMP), 4 (2.2%) suspicious for malignancy (SFM), and 1 (0.5%) malignant case. 156/178 patients (87.6%) had available follow-up data. The RON/ROM for cases with surgical follow-up were 33.3%(3/9) /22.2%(2/9) for non-diagnostic, 42.9%(9/21)/19%(4/21) for non-neoplastic, 76.5%(13/17)/29.4%(5/17) for AUS, 100.0%(2/2) /50.0%(1/2) for SUMP, and 100% (2/2) /100%(2/2) for the SFM category, respectively.

Conclusion: Applying MSRSGC on cystic salivary gland lesions improves patient management by preventing unnecessary surgery on non-neoplastic conditions. The ROM was highest in the SFM(100%), followed by the SUMP, AUS, non-diagnostic, and non-neoplastic categories. Less than adequate specimens may increase the AUS diagnosis.

Introduction

Fine needle aspiration (FNA) cytology is a well-accepted procedure for the pre-operative evaluation of salivary gland mass lesions [1]. Salivary gland FNA is minimally invasive and cost effective with a high accuracy in discriminating between non-neoplastic versus 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 non-neoplastic conditions [4].

Salivary gland neoplasms are uncommon, accounting only for 6% of all head and neck neoplasms and 0.3% of all malignancies in the United States [5]. Cystic salivary gland lesions are rare, accounting for up to 8% of all salivary gland lesions. However, a number of non-neoplastic and neoplastic processes may present as a cystic lesion [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 due to samples being 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 was suggested based upon the presence or absence of mucin, epithelium, and/or lymphocytes [6, 8]. However, previously there was no universal reporting system to report cytomorphologic findings in cystic salivary gland lesions and an associated risk of malignancy (ROM) and risk of neoplasia (RON). The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was published in 2018 [9], and is a risk-stratification classification scheme for which the ROM has been defined for each diagnostic category. Although data exists on the general performance of salivary gland FNA, less is known specifically about cystic salivary gland lesions and their impact based upon the MSRSGC. The diagnostic utility of pre-operative FNA cytology for cystic major

salivary gland lesions was evaluated in our previous study [10], but this was prior to adoption of the MSRSGC. Herein, the application of the MSRSGC to cystic salivary gland lesions was investigated and the ROM and RON were calculated for each diagnostic category.

Materials & Methods

The institutional review board of the Johns Hopkins Medical Institutions approved this study. Cases were identified by searching 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 pre-procedural ultrasound examination by a radiologist or by pre and post procedural palpation by an otolaryngologist-head and neck surgeon. For each case, the patient's age, sex, race, use of ultrasound guidance, and clinical non-surgical and surgical follow-up were recorded.

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

Non-Diagnostic: specimens consisted of non-mucinous cystic fluid only.

Non-neoplastic: 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 consist 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 cystic background where the differential diagnosis includes 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 (MN): Malignant cells in a cystic background such as keratinizing squamous cell carcinoma.

Diagnostic performance between FNA and follow-up data was recorded. Considering 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 with macrophages (cyst contents), squamous cells, oncocytes, and any atypical cells. The Milan diagnostic category and aforementioned prominent cytomorphologic features were correlated.

Data Analysis

ROM and RON were calculated for each diagnostic Milan category.

Risk of neoplasia= Number of neoplastic cases/total cases of interest.

Risk of malignancy= 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 (54%) males and 82 (46%) females with an average age of 53 years (range 4-90 years). There were 92 (51.7 %) patients that were Caucasian, 60 (33.8%) Black, 10 (1.8%) Asian, 2 (1.1%) Hispanic, and the race was unknown in 14 cases. FNA sites included the parotid gland (172, 96.6%), submandibular gland (4, 2.4%), and sublingual gland (1, 0.5%), as well as one specimen labeled “lymph node, level II” (1, 0.5%). Ultrasound guidance with on-site evaluation was utilized in 62 (34.8 %) cases **(Table 1).**

Follow-up

Clinical follow-up data was available for 154 (86.5 %) patients. Of these patients 87 (57.1 %) were initially managed conservatively with routine clinical evaluation and watchful waiting, 20 (13 %) underwent repeat FNA, and 46 (30 %) underwent surgical excision **(Figure 1)**. Additional studies were performed on 4 (20%) cases with repeat FNA, including core biopsies in three cases and flow cytometry in one case. The mean follow-up for the conservatively managed group was 10.5 years (ranging from 0.5

to 16.3) and 3 (3.4%) of these patients eventually underwent a repeat FNA after a mean follow-up of 6.2 years (ranging from 2.0 to 12.6). In addition, five (25 %) patients who initially received a repeat FNA underwent surgical excision after a mean time period of 4.3 years (ranging from 0.08 to 6.3).

Application of the Milan System for Reporting Salivary Gland Cytopathology

The MSRSGC was applied to all FNA cases for the purposes of this study. There were 52 (29.2%) non-diagnostic, 80 (44.9%) non-neoplastic, 35 (19.6%) AUS, 3 (1.7%) benign neoplasm, 3 (1.7%) SUMP, 4 (2.2%) SFM, and 1 (0.5%) malignant case(s). Of these 178 cases, clinical follow-up was available for 156 patients (87.6%) **(Figure 2).**

Surgical Follow-up

A total of 51/178 (28.6%) cases underwent surgical excision **(Table 1).** The majority of cases were diagnosed as non-neoplastic on FNA (n=21), followed by AUS (n=17), non-diagnostic (n=9), SUMP (n=2), and SFM (n=2). There were 14/51 cases (27.5%) that were diagnosed as malignant on surgical follow-up, including 6/30 (20%) FNA cases with non-neoplastic diagnoses, 5/17 (29.4%) FNA cases diagnosed as AUS, 1/2 (50%) cases diagnosed as SUMP, and 2/2 cases diagnosed as SFM (100%). Malignant diagnoses on surgical follow-up is shown for each category in Table 1. There were 15/51 cases (29.4%) that were diagnosed as benign neoplasms on surgical follow-up, which are listed for each category in Table 1. There were 19/51 cases (35.3%) that were diagnosed as a benign cyst on surgical follow-up, which are listed for each category in **Table 1.**

Subsequently, the prominent morphologic features comprising the cystic component (lymphocytes, mucin, acellular background debris containing scattered macrophages, squamous cells, oncocytes and atypical cells) were correlated with surgical follow-up. There were 21/59 (35.6%) cases containing numerous lymphocytes that had surgical follow-up. The benign diagnoses (n=16) included lymphoepithelial cyst (n=6), Warthin's tumor (n=4), apocrine hydrocystoma (n=1), branchial cleft cyst (n=1), ruptured cyst with squamous lining (n=1), benign lymphoid and fibrous tissue (n=1), cystadenoma with prominent lymphoid stroma (n=1) and parotid tissue with reactive lymphocytes and fibrosis (n=1). The malignant cases (n=5) consisted of cystic mucoepidermoid carcinoma (n=1), acinic cell carcinoma (n=1) , Epstein-Barr virus (EBV)-associated smooth muscle tumor (n=1), EBV-positive diffuse large B cell

lymphoma (n=1) and low-grade extranodal marginal zone lymphoma (MALT lymphoma, n=1). There were 7/12 (58.3%) cases with a prominent mucin component that had surgical follow-up, including five malignant and two benign cases reported as follows: mucoepidermoid carcinoma (n=5), oncocytic cystadenoma (n=1) and benign parotid tissue (n=1). Of the 74 cases with predominantly acellular background debris containing scattered macrophages twelve (12/74, 16.2%) had surgical follow-up, there were two malignancies, one benign neoplasm while the remainder (n=9) were non-neoplastic conditions. These diagnoses included papillary-cystic variant of acinic cell carcinoma (n=1), mucoepidermoid carcinoma (n=1), pleomorphic adenoma (n=1), branchial cleft cyst (n=1), retention cyst (n=1), benign cyst with oncocytic epithelium (n=1), chronic sialadenitis with duct dilation and oncocytic change (n=1), salivary duct cyst (n=4), and benign squamous lined cyst (n=1). There were 9/22 (40.1%) cases with prominent squamous cells with surgical follow-up including squamous cell carcinoma (n=2), Warthin's tumor (n=1), cystic sebaceous lymphadenoma (n=1), salivary duct cyst (n=3), epidermal inclusion cyst (n=1), and fibrous walled cyst with keratin debris and reactive atypia (n=1). Three cases with prominent oncocytes had conservative follow-ups. Of six cases with atypical cells, two (2/6, 33.3%) had surgical follow-ups including pleomorphic adenoma (n=1), and hemangioma (n=1).

The Milan categories and their associated prominent cytomorphologic findings

Non-Diagnostic category

The non-diagnostic category included 52 cases. The majority of these cases (n=47) showed acellular cystic fluid and scattered macrophages. Numerous lymphocytes were noted in four cases. Squamous cells were present in one case. Nine cases had surgical follow-up. A case with cyst contents containing numerous lymphocytes was a low grade cystic mucoepidermoid carcinoma (n=1). One case with background cyst contents with associated benign appearing acinar cells was diagnosed as acinic cell carcinoma, papillary cystic variant (n=1) on excision. Another case with cyst contents resulted as a pleomorphic adenoma with cystic change (n=1) on histology. The remaining cases on surgical follow-up included salivary duct cysts (n=3), retention cyst (n=1) and cyst with oncocytic epithelium (n=1). One case with prominent squamous cells was aspirated from a benign squamous cell lined cyst (n=1) on histology (**Figure3).**

Non-Neoplastic category

The non-neoplastic category included 80 cases. For cases placed within the non-neoplastic 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. Surgical follow-up on 12 cases with prominent lymphocytes were as follows: lymphoepithelial cyst (n=4), branchial cleft cyst (n=1), Warthin's tumor (n=3), EBV-associated smooth muscle tumor (n=1), apocrine hydrocystoma (n=1), and two lymphoma cases (EBV-positive diffuse large B cell lymphoma and low grade exteranodal marginal zone lymphoma). Surgical follow-up of cases with a 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 branchial cleft cyst (n=1). Six cases with prominent squamoid features had surgical follow-up including squamous cell carcinoma (n=1), epidermal inclusion cyst (n=1), cystic sebaceous lymphadenoma (n=1), and salivary duct cyst with squamous metaplasia (n=3).

AUS category

There were 35 cases included in this category. Predominant features that were identified include squamous cells (n=5), mucin (n=7), oncocytes (n=2), lymphocytes (n=5), atypical cells (n=5), and background cyst contents (n=4). Eight out of the twelve cases in this group with prominent lymphocytes had surgical follow-up, revealing diagnoses of Warthin's tumor (n=1) with focal squamous metaplasia, 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 out of seven of the cases with prominent mucin had surgical follow-up and were diagnosed as low-grade mucoepidermoid carcinoma (n=2), oncocytic cystadenoma (n=1), and benign parotid tissue (n=1). One out of four cases with cyst contents had surgical follow-up and was diagnosed as mucoepidermoid carcinoma (n=1). Two out of five cases with prominent squamous features had surgical follow-up including 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 out of five cases with atypical cells had surgical follow-up and were diagnosed as pleomorphic adenoma with florid metaplastic squamous differentiation and necrosis (n=1), Warthin's tumor (n=1), and hemangioma (n=1).

Benign Neoplasm category

There were three benign neoplasms identified. Cytologic examination showed prominent lymphocytes in two 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 three of these cases were followed by watchful waiting.

SUMP category

Three cases were included in the SUMP category. Two cases showed squamous epithelium and one case contained mucin. Two of these three cases had surgical follow-up. The case with mucin was diagnosed as a mucoepidermoid carcinoma (n=1) on surgical follow-up. One case with squamous epithelium was attributed to a Warthin's tumor (n=1) on surgical follow-up.

Suspicious for Malignancy category

There were four cases included in this category. All four cases contained mucin. In two of these cases a retention cyst was favored based on their cytologic and clinical findings. Two cases were diagnosed as mucoepidermoid carcinoma (n=2) on surgical follow-up.

Malignant category

The one 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 upon their prominent cytomorphologic findings

Surgical follow-up was available for 10 cases with prominent lymphocytes and the diagnoses based on histopathology were 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's tumor with squamous metaplasia (n=1), benign lymphoid tissue (n=1), and cystadenoma with prominent lymphoid hyperplasia (n=1). Seven cases with prominent mucin had surgical follow-up including five mucoepidermoid carcinomas (n=5), one oncocytic cystadenoma (n=1) and one case with just benign parotid tissue (n=1). Twelve cases with prominent acellular debris and few macrophages had surgical follow-up including acinic cell carcinoma (n=1), mucoepidermoid carcinoma (n=1), salivary duct cyst (n=4), pleomorphic adenoma (n=1), Warthin's tumor (n=1), benign cyst with oncocytic epithelium (n=1), chronic sialadenitis with duct dilation (n=1),

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 consisted of squamous cell carcinoma (n=2), salivary duct cyst (n=3), and one of each cystic sebaceous lymphadenoma (n=1), epidermal inclusion cyst (n=1), fibrous walled cyst with keratin debris and reactive atypia (n=1), and a Warthin's 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 the other group for all FNA cases with any follow-up including clinical and surgical. RON and ROM were tabulated for each category in **Tables 1 and 2**. RON in the non-neoplastic category for cases with surgical follow-up was 42.9% (9/21) and ROM 19% (4/21). Interestingly, ROM in the non-diagnostic category with surgical follow-up was 22.2% (2/9) which is higher than that in the non-neoplastic category proposed by the MSRSGC. RON and ROM for SFM were both 100% (2/2). The overall RON for all categories was 56.9% (29/51) and ROM was 27.5% (14/51), respectively. The overall RON and ROM were 18.8% (29/154) and 9.1% (14/154) respectively among all FNA cases with follow-up including clinical and surgical.

Discussion

The clinical presentation for a wide variety of salivary gland neoplastic and non-neoplastic 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 lesion are more complex on ultrasound and may contain variable degree of heterogeneity with internal debris or septations. However, these radiologic features are non-specific. 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 makes the interpretation of these cysts on imaging even more challenging. In addition, lymph nodes with involvement by metastatic carcinoma, lymphoma or even with reactive changes can mimic salivary gland cysts [14, 15]. As a result of these limitations by imaging, minimally invasive sampling of these lesions is

paramount in driving clinical decision-making and decreasing patients ultimately undergoing unnecessary surgical intervention. [4, 16]. Prior to the MSRSGC, salivary gland, cysts were evaluated based solely upon their cytomorphologic features such as the presence of lymphocytes, mucin or oncocytes [6]. The MSRSGC categorizes cysts into one of six tiers based upon their cytomorphologic findings. The non-neoplastic 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 employed for follow-up in cases with diagnoses of non-diagnostic, non-neoplastic and AUS. 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% (51/178) of cases had surgical follow-up. The ROM for non-diagnostic, non-neoplastic, benign, atypical, SUMP, SFM and malignant categories is 25%, 10%, 20%, <5%, 35%, 60%, and 90%, respectively according to the MSRSGC [9]. In the present study, the ROM for non-diagnostic, non-neoplastic, atypical, SUMP, and SFM was 22%, 19%, 29.4%, 50%, and 100%, respectively for cystic salivary gland lesions which is higher than those in all salivary gland lesions. Interestingly, ROM in the non-diagnostic category is slightly higher than that found for the non-neoplastic category, 22.2% (2/9) vs 19% (4/21), respectively, which it is not statistically significant. The ROM for non-neoplastic category is higher than the proposed percentage by the MSRSGC 19% (4/21) vs 10%, which can be explained by small number of cases with surgical follow-up in this category. The overall RON and ROM in cases with surgical follow-up were 56.9% (29/51) and 27.5% (14/51), respectively. One explanation for the higher ROM in cystic salivary gland lesions is that the majority of them were treated conservatively with clinical watchful waiting follow-up and occasionally repeat FNA, and that only cases with high clinical suspicion for malignancy underwent surgical intervention. The non-diagnostic category [17] and non-neoplastic category constituted the majority of cases in the MSRSGC. Of note, the non-diagnostic category is not equivalent to an insufficient specimen. Several studies have shown the significance of the cyst components mucin vs non-mucin, and also cellular features [6, 11]. Cellular and non-cellular components of all cystic lesions were recorded in this study including mucin, non-mucin cyst contents, lymphocytes, squamous cells, oncocytic cells and atypical cells. Cyst contents was the main component in the majority of non-diagnostic cases, while lymphocytes were the main cellular finding in the non-neoplastic category. Mucin was present in a subset of atypical, SUMP, and SFM cases, where all three of these categories are more often associated with malignancy. Mucoepidermoid carcinoma can present as a hypocellular mucinous cyst leading to a misdiagnosis [8]. Due to these observations, an FNA containing mucinous cyst fluid falls into the AUS category in the

MSRSGC. A non-mucin cystic component was predominantly seen in non-diagnostic cases in this study, whereas cases with notable lymphocytes were predominantly seen in non-neoplastic cases. The atypical category constituted aspirates with the most variable cellular and acellular components.

Surgical follow-up diagnoses in this study confirmed prior observations that non-neoplastic and neoplastic lesions, including both benign and malignant entities, may clinically manifest as a cyst [8, 18, 19]. Based on surgical follow-up, non-neoplastic conditions included salivary duct cyst, mucus retention cyst, chronic sialadenitis, lymphoepithelial cyst. Based on surgical follow-up benign neoplasms included pleomorphic adenoma, Warthin's tumor, and lymphadenoma. Based on 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 (HIV) - 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 the rendering a diagnosis of benign conditions such as Warthin tumor and intra-parotid lymph nodes. However, differentiating reactive lymphocytes from lymphomas is diagnostically challenging based upon cytomorphology alone without the use of ancillary studies. FNA of intraparotid lymph nodes are 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 with those in a cystic salivary gland [14, 15]. Squamous cells are another morphologic finding in FNA samples that can contribute to diagnostic difficulty of cystic salivary glands. They can be seen in both non-neoplastic and neoplastic lesions, including benign and malignant neoplasms. Epidermal inclusion cyst, salivary duct cyst with squamous cell metaplasia, and squamous cell carcinoma can all present as a cyst with variable degree of squamous cell atypia [7]. Oncocytes are considered a typical cellular component of Warthin's tumor and oncocytoma. However, oncocytes can also be seen in other non-neoplastic, metaplastic, and malignant conditions (e.g. Warthin-like variant of mucoepidermoid carcinoma) presenting diagnostic challenges [23, 24]. Our findings indicate that cystic salivary lesions may fall into different MSRSGC categories based on their cell type cytomorphology, quality and quantity. This does not imply 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 applicable to cystic salivary gland lesions, similar to solid salivary gland lesions. The surgical follow-up of several cases revealed entities that usually do not present as a cyst such as a pleomorphic adenoma, lymphomas, 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 MALT lymphoma case was associated with chronic sialadenitis and an adjacent 5.2 cm cyst. An EBV positive diffuse large B cell lymphoma, 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 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 123 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 depending upon 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. Whilst the numbers for SUMP and SFM cases in this study might not be statistically significant, they are informative and can be utilized in future studies Such as meta-analysis studies. The ROM and RON of all categories might be impacted by the fact that there are relatively few numbers of cases in each category. However, this study clearly shows that application of the MSRSGC on the cystic salivary gland lesion guided the clinicians toward a more conservative approach and prevented unnecessary surgery in a substantial number of cases.

In conclusion, FNA is a minimally invasive and cost effective diagnostic tool to help 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 lesion which may prevent unnecessary surgery 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 non-diagnostic category, followed by non-neoplastic aspirates. The ROM in cystic salivary gland lesions of all categories with surgical follow-up is slightly higher than those proposed by the MSRSGC, and likely attributed to the fact that only cases with a high suspicion for malignancy undergo surgical intervention. However, RON and ROM fall within the MSRSGC proposed range when calculated for all FNA cases with any follow-up including clinical and surgical.

References

1. Eytan DF, Yin LX, Maleki Z, et al. Utility of preoperative fine needle aspiration in parotid lesions. Laryngoscope. 2018;128: 398-402.

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. 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.

4. Layfield LJ, Gopez E, Hirschowitz S. Cost efficiency analysis for fine-needle aspiration in the workup of parotid and submandibular gland nodules. Diagn Cytopathol. 2006;34: 734-738.

5. Eveson JW AP, Gnepp DR, El-Naggar AK. Tumours of the salivary glands: Introduction. In: Barnes L, Eveson JW, Reichart P, Sidransky D, editors. World Health Organization Classification of Tumours. Pathology & Genetics Head and Neck Tumours. Lyon: IARCPress, 2005.

6. Faquin WC PCCamlMal-gmcIFW, Powers CN, editors. Salivary gland cytopathology. Boston, MA: Springer US; 2008:159–181.

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. Layfield LJ, Gopez EV. Cystic lesions of the salivary glands: cytologic features in fine-needle aspiration biopsies. Diagn Cytopathol. 2002;27: 197-204.

9. Faquin WC RE, Editors, Baloch, Z, Barkan, G.A., Maria P. Foschini, M.P., Kurtycz, D.F.I., Pusztaszeri, M., Vielh, P. Assoc. Editors, The Milan System for Reporting Salivary Gland Cytopathology, 2018. Springer Press, Cham Switzerland, ISBN 978-3-319-71284-0, https://doi.org/10.1007/978-3-319-71285-7.

10. Allison DB, McCuiston AM, Kawamoto S, Eisele DW, Bishop JA, Maleki Z. Cystic major salivary gland lesions: Utilizing fine needle aspiration to optimize the clinical management of a broad and diverse differential diagnosis. Diagn Cytopathol. 2017;45: 800-807.

11. Pantanowitz L, Thompson LDR, Rossi ED. Diagnostic Approach to Fine Needle Aspirations of Cystic Lesions of the Salivary Gland. Head Neck Pathol. 2018;12: 548-561.

12. Bialek EJ, Jakubowski W. Mistakes in ultrasound examination of salivary glands. J Ultrason. 2016;16: 191-203.

13. Bialek EJ, Jakubowski W, Zajkowski P, Szopinski KT, Osmolski A. US of the major salivary glands: anatomy and spatial relationships, pathologic conditions, and pitfalls. Radiographics. 2006;26: 745-763.
14. Ahuja AT, Ying M, Yuen HY, Metreweli C. 'Pseudocystic' appearance of non-Hodgkin's lymphomatous nodes: an infrequent finding with high-resolution transducers. Clin Radiol. 2001;56: 111-115.
15. Kessler A, Rappaport Y, Blank A, Marmor S, Weiss J, Graif M. Cystic appearance of cervical lymph nodes is characteristic of metastatic papillary thyroid carcinoma. J Clin Ultrasound. 2003;31: 21-25.
16. Carrillo JF, Ramirez R, Flores L, et al. Diagnostic accuracy of fine needle aspiration biopsy in preoperative diagnosis of patients with parotid gland masses. J Surg Oncol. 2009;100: 133-138.
17. Kim MW, Kim DW, Jung HS, et al. Factors Influencing the Outcome of Ultrasound-Guided Fine-Needle Aspiration for Salivary Gland Lesion Diagnosis. J Ultrasound Med. 2016;35: 877-883.
18. Takita H TT, Shimono T, Tanaka H, Iguchi H, Hashimoto S, Kuwae Y, Ohsawa M, Miki Y. Cystic lesions of the parotid gland: radiologic-pathologic correlation according to the latest World Health Organization 2017 Classification of Head and Neck Tumours. Jpn J Radiol. 2017;35:629–47.
19. Ellis GL AP, editors. Tumors of the salivary glands. AFIP atlas of tumor pathology, 4th series, fascicle 9. Silver Spring, MD: ARP Press; 2008.
20. Upile T, Jerjes W, Al-Khawalde M, et al. Branchial cysts within the parotid salivary gland. Head Neck Oncol. 2012;4: 24.
21. Martinoli C, Pretolesi F, Del Bono V, Derchi LE, Mecca D, Chiaramondia M. Benign lymphoepithelial parotid lesions in HIV-positive patients: spectrum of findings at gray-scale and Doppler sonography. AJR Am J Roentgenol. 1995;165: 975-979.
22. Hughes JH, Volk EE, Wilbur DC, Cytopathology Resource Committee CoAP. Pitfalls in salivary gland fine-needle aspiration cytology: lessons from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. Arch Pathol Lab Med. 2005;129: 26-31.
23. Hang JF, Shum CH, Ali SZ, Bishop JA. Cytological features of the Warthin-like variant of salivary mucoepidermoid carcinoma. Diagn Cytopathol. 2017;45: 1132-1136.
24. Wade TV, Livolsi VA, Montone KT, Baloch ZW. A cytohistologic correlation of mucoepidermoid carcinoma: emphasizing the rare oncocytic variant. Patholog Res Int. 2011;2011: 135796.
25. Moatamed NA, Naini BV, Fathizadeh P, Estrella J, Apple SK. A correlation study of diagnostic fine-needle aspiration with histologic diagnosis in cystic neck lesions. Diagn Cytopathol. 2009;37: 720-726.

Figure legends

Figure 1. A diagram of application of the Milan System for Reporting Salivary Gland Cytopathology to cystic salivary gland lesions and their follow-up.

Figure 2. A pie chart shows distribution of cases in each Milan System category.

Figure 3. Shows the distribution of prominent cytomorphologic findings of each Milan system categories.

Figure 4a. FNA of a 2 cm cystic submandibular mass consisted of scattered macrophages and lymphocytes in a background of mucin which is an example of atypia of undetermined significance (Diff-Quik stain, X200).

Figure 4b. Surgical follow-up of the case showed mucocele with granulation tissue and extravasated mucin (H&E, X100).

Figure 5a. FNA of a 4 cm cystic parotid mass exhibited epithelial fragments with basaloid features and scant stroma which was categorized as SUMP (Papanicolaou stain, X200).

Figure 5b. Surgical follow-up was diagnosed as lymphadenoma (H&E, X100).

Figure 6a. FNA of a 3 cm left parotid mass comprised of fragments of epithelial cells in a background of mucin suspicious for malignancy (Diff-Quik stain, X200).

Figure 6b. Surgical follow-up was consistent with an intermediate grade mucoepidermoid carcinoma (H&E stain, X100).

MSRSGC	Gender Age (years)	FNA Site	Ultrasound guidance FNA	Surgical follow-up	RON (%) ROM (%)
Non-diagnostic (9/52)	Male (5) Female (4) 4-85 (51.9)	Parotid (9)	Yes (2) No (8)	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)
Non-neoplastic (21/80)	Male (13) Female (8) 4-87 (52.1)	Parotid (21)	Yes (8) No (12)	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)	42.9% (9/21) 19% (4/21)

				Lymphoma (2)	
AUS (17/35)	Male (11) Female (6) 10-84 (53.7)	Parotid (16) Lymph node Level IIB	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)	Male (1) Female (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)	Male (1) Female (1) 15-82 (48.5)	Parotid (2)	No (2)	Mucoepidermoid carcinoma (2)	100% (2/2) 100% (2/2)
Total (51)	Male (31) Female (20) 4-87 (53)	Parotid (50) Lymph node (1)	Yes (20) No (31)	Non-neoplastic (22) Benign neoplasm (15) Malignant neoplasm (14)	56.9% (29/51) 27.5% (14/21)

Table 1. The salivary gland FNA cases categorized based upon the Milan system for reporting salivary gland cytopathology and are correlated with their surgical follow-up. Risk of malignancy and risk of neoplasm calculated only on FNA cases with surgical follow-up.

Footnote: AUS; atypia of undetermined significance, SUMP; salivary gland neoplasm of uncertain malignant potential, RON; risk of neoplasm, ROM; risk of malignancy.

MSRSGC	Cases with surgical follow-up only		All cases with follow-up	
	RON (%)	ROM (%)	RON (%)	ROM (%)
Non-diagnostic (n=48)	33.3% (3/9)	22.2% (2/9)	6.25% (3/48)	4.2% (2/48)
Non-neoplastic (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)	66.6% (2/3)	66.6% (2/3)
Total (n=154)	56.9% (29/51)	27.5% (14/51)	18.8% (29/154)	9.1% (14/154)

Table 2. There is a difference in risk of neoplasm and risk of malignancy when only cases with surgical follow-up are included vs the time that all cases with follow-up are included.

Abbreviation: AUS; atypia of undetermined significance, SUMP; salivary gland neoplasm of uncertain malignant potential, RON; risk of neoplasia, ROM; risk of malignancy.

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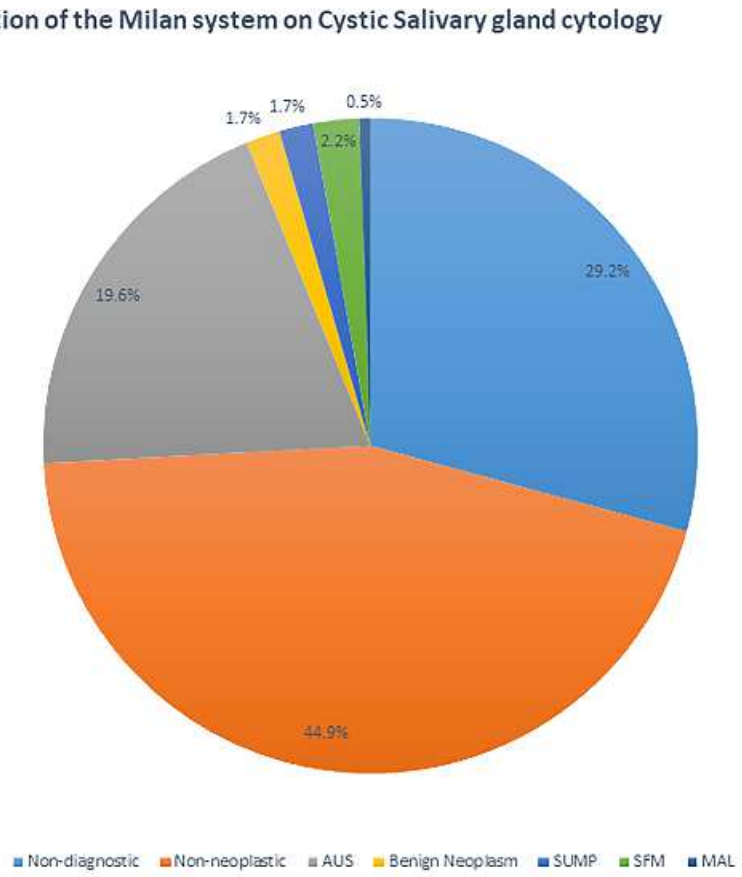

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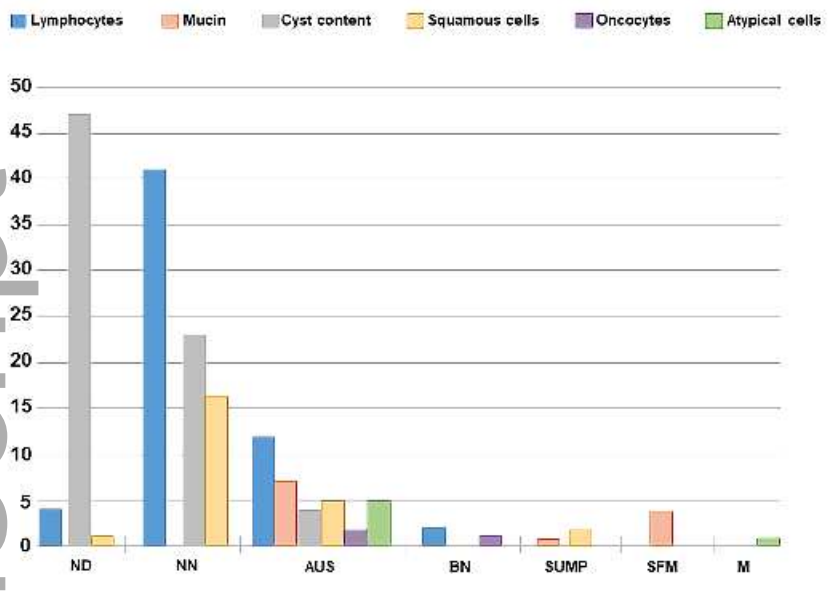

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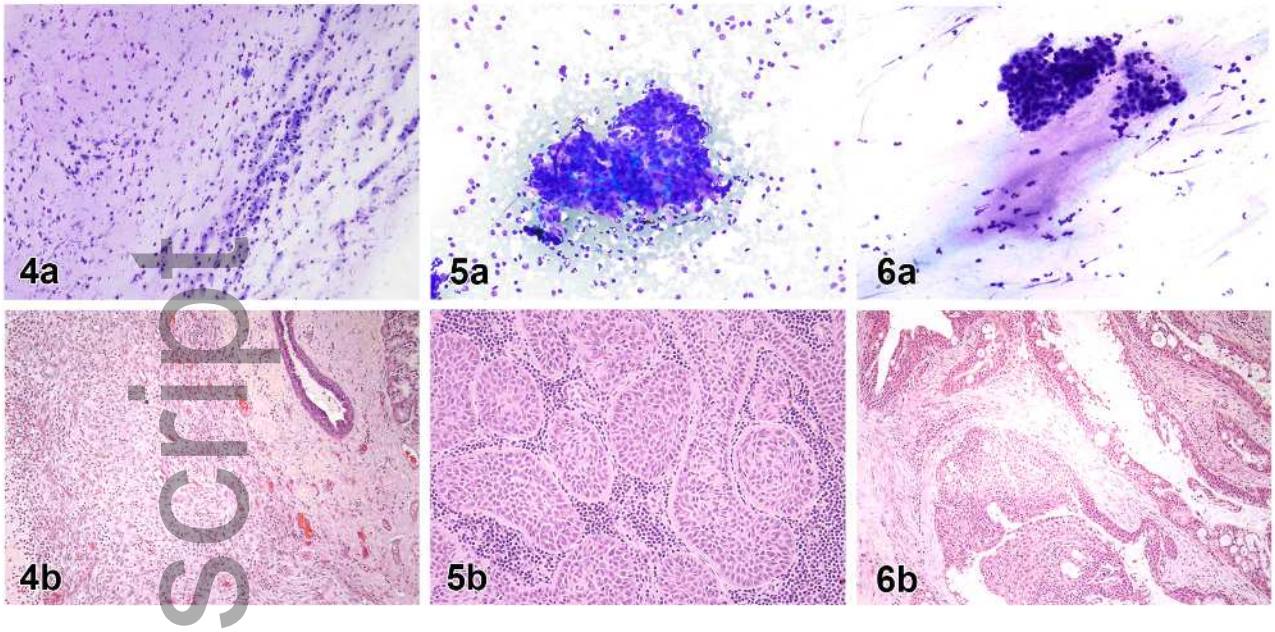

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