

Interventional cytopathologist perspective on the Milan System Classification: a study on 929 consecutive salivary gland fine-needle aspirations with a focus on challenging diagnostic categories

Anna Maria Carillo¹, Isabella Sorice¹, Maria Salatiello¹, Rosa Maria Di Crescenzo², Pasquale Pisapia¹, Giovanni Dell'Aversana Orabona³, Elena Vigliar¹, Stefania Staibano², Giancarlo Troncone¹, Claudio Bellevicine¹

¹ Department of Public Health, University of Naples Federico II, Naples, Italy; ² Department of Advanced Biomedical Sciences, University of Naples, Italy; ³ Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples Federico II, Naples, Italy

Summary

Background. Although the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) has improved the diagnosis and management of salivary gland lesions, determining the risk of malignancy (ROM) for AUS and SUMP categories remains challenging. We investigated the role of interventional cytopathologists in refining the differential diagnosis of these categories.

Methods. We searched for salivary gland fine-needle aspirations (FNAs) performed at our Institution since the publication of the first edition of MSRSGC. In our Institution, salivary gland FNAs are performed by interventional cytopathologists only. We checked for the availability of histopathology reports to calculate the risk of neoplasm (RON) and ROM. Sensitivity, specificity, negative predictive value, and positive predictive values of our FNAs were assessed by focusing on the contribution of the AUS and SUMP categories to our diagnostic accuracy.

Results. 929 salivary gland FNA diagnoses were retrieved. 37.02% FNAs had an available surgical follow-up. The ROM for each category was: 6% (ND); 0 (NN); 15.15% (AUS); 1.14% (NB); 24.4% (SUMP); 66.7% (SFM); and 94.74% (M). We observed a high level of concordance between our ROM data and the values proposed by the MSRSGC; higher accuracy (93.17%) and sensitivity (97%) were obtained when the AUS category was considered as a positive index for detecting salivary neoplasms; the best diagnostic accuracy (93.33%) was obtained when the SUMP category was considered as a negative index for malignancy.

Conclusion. Interventional cytopathologists play an important role in salivary gland cytopathology, as demonstrated by the overt concordance between our ROM rates and those recommended by the MSRSGC.

Key words: FNA, salivary gland, MSRSGC, cytopathology, interventional cytopathology

Background

Ultrasound (US) guided fine-needle aspiration (FNA) is a safe, minimally invasive, and cost-effective technique for preoperative diagnosis and management of salivary gland lesions ¹. However, FNA diagnostic performance may be compromised by several cytologic confounding factors, including a lack of architecture, significant heterogeneity, and frequent cytomorphologic overlap between salivary gland lesions ².

Received: November 18, 2024
Accepted: December 10, 2024

Correspondence

Giancarlo Troncone
E-mail: giancarlo.troncone@unina.it

How to cite this article: Carillo AM, Sorice I, Salatiello M, et al. Interventional cytopathologist perspective on the Milan System Classification: a study on 929 consecutive salivary gland fine-needle aspirations with a focus on challenging diagnostic categories. *Pathologica* 2024;116:358-367. <https://doi.org/10.32074/1591-951X-N815>

© Copyright by Società Italiana di Anatomia Patologica e Citopatologia Diagnostica, Divisione Italiana della International Academy of Pathology



OPEN ACCESS

This is an open access journal distributed in accordance with the CC-BY-NC-ND (Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International) license: the work can be used by mentioning the author and the license, but only for non-commercial purposes and only in the original version. For further information: <https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>

Moreover, until recently, its diagnostic value for salivary gland lesions was limited by the lack of a univocal reporting system. Accordingly, concerted efforts by an international panel of experts created the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC). This system provides a tiered system that correlates the ROM with each diagnostic category, whilst recommending the best treatment option for each category³. This system, that has recently been slightly revised⁴, includes 6 diagnostic categories: I) non-diagnostic (ND), II) non-neoplastic (NN), III) atypia of undetermined significance (AUS), IV) neoplasm – further sub-classified into IV A neoplasm – benign (NB) and IV B neoplasm – salivary gland neoplasm of uncertain malignant potential (SUMP) – V) suspicious for malignancy (SFM), and VI) malignant (M).

Most FNAs are classified as NB, a category that includes Warthin tumors (WTs) and pleomorphic adenomas (PA) which are the most prevalent benign salivary gland tumors⁵. The general treatment for these patients entails either a complete resection of the tumor with a narrow excision of normal tissue to prevent recurrence or a nerve-preserving parotidectomy. However, when NB is diagnosed on an FNA performed on a submandibular gland, a supra-fascial plane resection is recommended⁶. Conversely, the SFM or the M categories are associated with high ROM. For these patients, cross-sectional imaging, as well as low versus high-grade differential diagnosis, is recommended to plan the extent of surgery and the likelihood of neck dissection.

The NN category is usually managed clinically unless worrisome imaging and clinical findings suggest an “out-of-target” FNA procedure. In this case, a repeat FNA is recommended⁴.

While the diagnosis of the above-mentioned categories is rather straightforward, the ND, AUS, and SUMP categories remain the most challenging. A major reason is that they are not readily actionable. In fact, repeated FNAs (or a core biopsy in selected cases) are required after diagnosis of ND and AUS⁷. Even more complicated is the SUMP category, since it includes benign and malignant salivary gland neoplasms with overlapping cytomorphological features. Although the MSRSGC proposes 35% ROM for this category, the management of these patients is difficult to define without ancillary methods⁸. Accordingly, the MSRSGC has suggested a morphological sub-classification of the SUMP category, which identifies the basaloid subclass as the one bearing the highest ROM⁹. Moreover, the MSRSGC also recommends using the intraoperative frozen section technique to refine the histopathological diagnosis during surgery.

Since 1976, our institution has provided a cytopathol-

ogist-performed FNA service for the diagnosis of different types of lesions, including thyroid, lymph nodes, breast, and salivary glands¹⁰. In 2018, after the publication of the first edition of the MSRSGC, we adopted this classification scheme in our routine daily practice to standardize the descriptive diagnoses previously adopted for salivary gland FNA reports.

The aim of this study was to validate the diagnostic utility of MSRSGC in diagnosing salivary gland tumors through cytopathologist-performed FNA. To this aim, we investigated the diagnostic experience of our institution in the first 6 years following the adoption of the MSRSGC system by focusing on the three most diagnostically challenging categories, namely, ND, AUS, and SUMP.

Methods

We searched our electronic database for salivary gland FNAs performed from February 2018–February 2024 and classified with MSRSGC. Clinical data such as sex, age, location, and available final histologic diagnosis were also collected from our database when available. All FNAs were performed with 23-gauge needles by an experienced interventional cytopathologist under US-guidance. In selected cases, at least one smear was air-dried and stained with Diff-Quik to enable rapid on-site evaluation (ROSE). After the ROSE, the interventional cytopathologist performed additional passes in order to obtain either Papanicolaou stained smears or cell-blocks for ancillary staining. The availability of histological reports was searched for each patient to calculate the risk of neoplasm (RON) and risk of malignancy (ROM). However, considering that RON and ROM can be overestimated in categories not requiring surgery, namely, ND, NN, and AUS categories¹¹, we also calculated the *overall risk of neoplasm* (ORON = number of neoplasms on surgical follow-up for that cytological category / overall number of cases classified in that cytological category) and the *overall risk of malignancy* (OROM = number of malignant neoplasms on surgical follow-up for that cytological category / overall number of cases classified in that cytological category). This was done on the assumption that patients with no histological records were still undergoing follow-up care without further clinical or imaging data suggesting a malignant neoplasm. To calculate the impact of MSRSGC on our diagnostic practice, we also calculated sensitivity, specificity, negative predictive values (NPV), and positive predictive values (PPV) of our cytopathologist-performed FNAs. In particular, we evaluated how AUS and SUMP diagnoses correlated with

diagnostic accuracy of our salivary gland FNAs. For this analysis, we considered two scenarios: 1) the accuracy of MSRSGC in distinguishing non-neoplastic from neoplastic lesions (scenario 1) and benign from malignant neoplasms (scenario 2). The analysis included only those FNA cases that were satisfactory for cytologic evaluation.

Written informed consent was obtained from all patients, in accordance with the general authorization to process personal data for scientific research purposes from "The Italian Data Protection Authority" (<http://www.garanteprivacy.it/web/guest/home/docweb/-/docwebdisplay/export/2485392>). All information regarding human material was managed using anonymous numerical codes, and all samples were handled in compliance with the Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>) The data that support the findings of this study are available from the corresponding author, G.T., upon reasonable request.

Results

A total of 929 FNA diagnosis of salivary gland lesions were retrieved; the cases showed a male predominance (M:F = 1.1:1) and a mean age of 59 years (range 7-92). The anatomical location was reported in 95.26% of cases (n = 884/929 cases), with the majority of FNAs (743/884, 84.05%) taken from the parotid glands. In 552 cases (59.42%), the mean size of the lesions was 22.25 mm (range 1.7-80 mm). According to the MSRSGC system, we classified 17.55% (n = 163) cases as ND, 15.50% (n = 144) as NN, 11.09% (n = 103) as AUS, 41.77% (n = 388) as NB, 6.14% (n = 57) as SUMP, 2.37% (n = 22) as SFM, and 5.49% (n = 51) as M. The distribution of these categories is summarized in Table I. A CB was available in n = 60

(6.49% of all FNAs) cases. In particular, the majority of CBs were performed in M (n = 20/60, 33.33%) cases.

Out of 929 FNAs, 37.02% (n = 344) cases had an available surgical follow-up, in particular 14.53% ND (n = 50), 4.65% NN (n = 16), 9.59% AUS (n = 33), 51.16% NB (n = 176), 11.91% SUMP (n = 41), 2.61% SFM (n = 9), and 5.52% M (n = 19). Histological follow-ups included: 14.82% (n = 51/344) non-neoplastic lesions (NNs), 72.38% (n = 249) benign neoplasms, and 12.79% (n = 44) malignant neoplasms.

In Table I, the ROM for each MSRSGC category is reported and compared with that proposed by the first and second edition of MSRSGC; RON and ORON are also reported. In Table II the histopathological diagnoses of the available surgical outcomes are detailed.

Briefly, for the ND category, the RON was 46% and the ROM was 6%, whereas the ORON and OROM were 14.1% and 1.84%, respectively (Tab. I).

Most of the ND category comprised ND cystic cases (61.35%, n = 100/160). As shown in Table II, 35% of all ND cystic FNAs had surgical follow-up (n = 35); the RON was 42.86% and the ROM was 5.71%; the ORON and OROM were respectively 15% and 2%, respectively.

For the NN category, the RON was 43.7%, whereas the ORON was 4.86%; no malignant neoplasms were retrieved among the available surgical follow-ups (Tabs. I, II).

For the AUS category, the RON was 69.7%, and the ROM was 15.15%; the ORON and OROM were 22.3% and 4.85%, respectively. The most common benign neoplasm classified as AUS was Warthin Tumor (WT) (n = 11/18, 61.1%), whereas the most frequent malignant one was MEC (n = 3/5, 60%) (Tab. II).

The NB category showed a very high RON (98.3%), but a very low ROM (1.14%). In particular, the most common neoplasm in the NB category was WT (n = 99/176, 56.25%), followed by PA (n = 65/176,

Table I. RON, ROM, ORON and OROM of FNA with available follow-up, along with follow-up recommended by the two editions of MSRSGC.

MSRSGC categories	Total	Total With FU	NN	NB	NM	RON	ORON	ROM	OROM	ROM in I edition of MSRSGC	ROM in II edition of MSRSGC
ND (I)	163	50	27	20	3	46%	14.1%	6%	1.84%	25%	15%
NN (II)	144	16	9	7	0	43.7%	4.86%	0	0	10%	11%
AUS (III)	103	33	10	18	5	69.7%	22.3%	15.15%	4.85%	20%	30%
NB (IV A)	388	176	3	171	2	98.3 %		1.14%		< 5%	< 3%
SUMP (IV B)	57	41	2	29	10	95.1%		24.4%		35%	35%
SFM (V)	22	9	0	3	6	100%		66.7%		60%	83%
M (VI)	51	19	0	1	18	100%		94.74%		90%	98%

Legend: RON = risk of neoplasm; ROM = risk of malignancy; ORON = overall risk of neoplasm; OROM = overall risk of malignancy.

Table II. Histological diagnoses available for each MSRSGC category.

I ND (15 cases, 23.81% of total ND)	- <u>Non-neoplastic</u> (7 cases, 46.7%): lymph node (1 case, 14.3%), inflammation (1 case, 14.3%), cyst (3 cases, 42.8%), sialolithiasis (1 case, 14.3%), normal gland (1 case, 14.3%) - <u>Neoplasm Benign</u> (7 cases, 46.7%): WT (3 cases, 42.9%), lipoma (1 case, 14.3%), myoepithelioma (1 case, 14.3%), PA (1 case, 14.3%), neurofibroma (1 case, 14.3%) - <u>Neoplasm Malignant</u> (1 case, 6.7%): carcinosarcoma (1 case, 100%)
I ND cyst (35 cases, 35% of total ND cyst)	- <u>Non-neoplastic</u> (20 cases, 57.14%): cyst (17 cases, 85%), mucocoele (1 case, 5%), inflammation (1 case, 5%), lymph node (1 case, 5%) - <u>Neoplasm Benign</u> (13 cases, 37.14%): WT (9 case, 69.2%), cystadenoma (2 cases, 15.4%), PA (1 case, 7.7%); neurofibroma (1 case, 7.7%) - <u>Neoplasm Malignant</u> (2 cases, 5.71%): acinic cell carcinoma (1 case, 50%), lymphoma (1 case, 50%)
II NN (16 cases, 11.1% of total NN)	- <u>Non-neoplastic</u> (9 cases, 56.25%): fibrosis (2 cases, 22.2%), reactive hyperplasia (1 case, 11.1%), sialolithiasis (2 cases, 25%), normal gland (1 case, 11.1%), cyst (1 case, 11.1%), chronic sialadenitis (2 cases, 22.2%) - <u>Neoplasm benign</u> (7 cases, 43.75%): WT (3 cases, 42.8%), lipoma (1 case, 14.28%), oncocytoma (1 case, 14.28%), PA (1 case, 14.28%), neurofibroma (1 case, 14.28%) - <u>Neoplasm malignant</u>: 0
III AUS (33 cases, 32.03% of total AUS)	- <u>Non-neoplastic</u> (10 cases, 30.3%): chronic inflammation (3 cases, 30%) acute sialadenitis (3 cases, 30%), branchial cyst (1 case, 10%), salivary duct cyst (1 case, 10%), normal gland (1 case, 10%), ectopic thyroid tissue (1 case, 10%) - <u>Benign neoplasms</u> (18 cases, 54.5%): WT (11 cases, 61.1%); PA (3 cases, 16.67%); schwannoma (1 case, 5.55%); myoepithelioma (1 case, 5.55%); oncocytoma (2 cases, 11.1%) - <u>Malignant neoplasms</u> (5 cases, 4.85%): squamous cell carcinoma (1 case, 20%); mucoepidermoid carcinoma (3 cases, 60%); carcinosarcoma (1 case, 20%)
IV A NB (176 cases, 45.36% of total NB)	- <u>Non-neoplastic</u> (3 cases, 1.7%): inflammation (1 case, 33.3%), normal gland (1 case, 33.3%), metaplasia (1 case, 33.3%) - <u>Neoplasm benign</u> (171 cases, 97.16%): PA (66 cases, 38.6%), WT (99 cases, 57.8%), oncocytoma (3 cases, 1.75%), myoepithelioma (1 case, 0.58%), lipoma (1 case, 0.58%) - <u>Neoplasm malignant</u> (2 cases, 1.14%): basal cell carcinoma (1 case, 50%), carcinoma ex pleomorphic adenoma (1 case, 50%)
IV B SUMP (41 total cases, 71.9% of total SUMP)	- <u>Non-neoplastic</u> (2 cases, 4.87%): nodular oncocytic hyperplasia (1 case, 50%), chronic sialadenitis (1 case, 50%) - <u>Benign neoplasms</u> (29 cases, 70.7%): PA (15 cases, 51.72%); basal cell adenoma (6 cases, 20.7%); paraganglioma (1 case, 3.45%); myoepithelioma (5 cases, 17.24%); oncocytoma (1 case, 3.45%); WT (1 case, 3.45%) - <u>Malignant neoplasms</u> (10 cases, 24.4%): acinic cell carcinoma (5 cases, 50%); adenoid cystic carcinoma (1 case, 10%); carcinoma ex pleomorphic adenoma (1 case, 10%); secretory carcinoma (1 case, 10%); mucoepidermoid carcinoma (1 case, 10%), epithelial-myoepithelial carcinoma (1 case, 10%)
V SFM (9 total cases, 41% of total SFM)	- <u>Non-neoplastic</u> (0 cases) - <u>Benign neoplasms</u> (3 cases, 33.3%): WT (1 case, 33.3%); myoepithelioma (2 cases, 66.6%) - <u>Malignant neoplasms</u> (6 cases, 66.6%): mucoepidermoid carcinoma (1 case, 16.7%); carcinosarcoma (1 case, 16.7%); lymphoepithelial carcinoma (1 case, 16.7%); sarcoma (1 case, 16.7%); acinic cell carcinoma (1 case, 16.7%); pilomatrix carcinoma (1 case, 16.7%)
VI M (19 total cases, 37.25% of total M)	- <u>Non-neoplastic</u> (0 cases) - <u>Neoplasm benign</u> (1 case, 5.26%): PA (1 case, 100%) - <u>Neoplasm malignant</u> (18 cases, 94.7%): ductal cell carcinoma (1 case, 5.56%), lymphoma (2 cases, 11.1%), mucoepidermoid carcinoma (2 cases, 11.1%), myoepithelial carcinoma (1 case, 5.56%), renal cell carcinoma (1 case, 5.56%), squamous cell carcinoma (8 cases, 44.4%), malignant solitary fibrous tumor (1 case, 5.56%), secretory carcinoma (1 case, 5.56%)

Legend: WT = Warthin tumor; ME = myoepithelioma; PA = pleomorphic adenoma; AcicC = acinic cell carcinoma; SCC = squamous cell carcinoma; MEC = mucoepidermoid carcinoma; BCAC = basal cell adenocarcinoma; CXPA = carcinoma ex pleomorphic adenoma; BCA = basal cell adenoma; AdCC = adenoid cystic carcinoma; SC = secretory carcinoma; EMC = epithelial-myoepithelial carcinoma; MECA = myoepithelial carcinoma; RCC = renal cell carcinoma.

36.93%) (Tab. II).

For the SUMP category, the RON was as high as that shown by the NB category (95.1%), but the ROM (24.4%) was lower; nonetheless it was below the 35% ROM recommended by both editions of MSRSGC. The most frequent malignant neoplasm of the SUMP category was acinic cell carcinoma (AcicC) (n = 5

out of 10 malignant neoplasms classified as SUMP, 50%). Conversely, the most frequent benign neoplasm was PA (n = 15 out of 29 benign neoplasms classified as SUMP, 51.72%). In addition, we divided SUMP FNAs with surgical follow-ups into three subcategories (basaloid, clear cell, and oncocytic), when the subcategorization reported in the second edition

Table III. SUMP subtypes with histologic follow-up.

Subtypes	Histology	RON	ROM
<u>Basaloid</u> (n = 28, 70%; n = 23 with surgical fu, 82.14%)	- Chronic sialadenitis (n = 1, 4.35%) - BCA (n = 5, 21.7%) - PA (n = 11, 47.8%) - ME (n = 2, 8.7%) - AciCC (n = 1, 4.35%) - AdCC (n = 1, 4.35%) - CXPA (n = 1, 4.35%) - EMC (n = 1, 4.35%)	95.65% (n = 22/23)	17.39% (n = 4/23)
<u>Clear cell</u> (n = 6, 15%; n = 5 with surgical fu, 83.3%)	- AciCC (n = 3, 60%) - SC (n = 1, 20%) - MEC (n = 1, 20%)	100% (n = 5/5)	100% (n = 5/5)
<u>Oncocytic</u> (n = 5, 12.5%, all with surgical fu)	- Nodular oncocytic hyperplasia (n = 1, 20%) - Oncocytoma (n = 1, 20%) - ME (n = 1, 20%) - WT (n = 1, 20%) - PA (n = 1, 20%)	80% (n = 4/5)	60% (n = 3/5)

Legend: BCA = basal cell adenoma; PA = pleomorphic adenoma; ME = myoepithelioma; AciCC = acinic cell carcinoma; AdCC = adenoid cystic carcinoma; CXPA = carcinoma ex pleomorphic adenoma; EMC = epithelial-myoepithelial carcinoma; ME = myoepithelioma; SC = secretory carcinoma; MEC = mucoepidermoid carcinoma; WT = warthin tumor.

Table IV. Diagnostic performance of salivary gland FNA in detecting neoplasms.

Scenario	Sensitivity	Specificity	PPV	NPV	Accuracy
1	88.89%	79.17%	97.96%	38.78%	88.10%
2	97%	37.50%	95.10%	56.25%	93.17%

Scenario 1: nonneoplastic and AUS as a negative index test result for neoplasm, NB, SUMP, SFM, M as a positive test result for neoplasm.
Scenario 2: nonneoplastic as a negative index test result for neoplasm; AUS, NB, SUMP, SFM, M as a positive test result for neoplasm.

Table V. Diagnostic performance of salivary gland FNA in detecting malignant neoplasms.

Scenario	Sensitivity	Specificity	PPV	NPV	Accuracy
1	94.44%	83.82%	50.75%	98.84%	85.42%
2	66.67%	98.04%	85.71%	94.34%	93.33%

Scenario 1. NB as a negative index test result for malignancy; SUMP, SFM, M as a positive test result for malignancy.
Scenario 2: NB and SUMP as a negative index test result for malignancy; SFM, M as a positive test result for malignancy.

of the MSRSGC was clearly stated in the diagnostic reports (n = 33/57 SUMP FNAs, 57.90%)⁴. The highest ROM (100%) was seen in the clear cell subcategory (Tab. III).

Malignant neoplasms were correctly classified as either SFM or M in most cases; in particular, 100% RON was seen for both categories, whereas 66.7% ROM was seen for the SFM category, and 94.74% ROM was seen for the M category. The most common malignancy was mucoepidermoid carcinoma (MEC) (Tabs. II, III). Immunocytochemical stainings performed in M cases were helpful to further subtype either primary neoplasm such as secretory carcinoma (SC), salivary duct carcinoma (SDC) and mucoepidermoid carcinoma (MEC) and metastatic diseases (squamous cell carcinoma, renal cell carcinoma etc.).

Out of n = 51 malignant cases categorized into the M category, n = 20 (39.22%) had concurrent cell blocks on which ancillary techniques were performed (see Supplementary Table).

The sensitivity, specificity, PPV, NPV, and accuracy of the MSRSGC, according to two different clinical scenarios, are summarized in Tables IV and V.

In scenario 1 (non-neoplastic vs neoplastic, Tab. IV), given the intermediate RON and low ROM observed for the AUS category, we considered this category as either a negative index; i.e., FNAs classified as AUS were expected to be non-neoplastic (Tab. IV, row 1) or a positive index; i.e., FNAs classified as AUS were expected to be neoplastic (Tab. IV, row 2). When the AUS category was considered as a positive index, we obtained the highest diagnostic accuracy (93.17%,

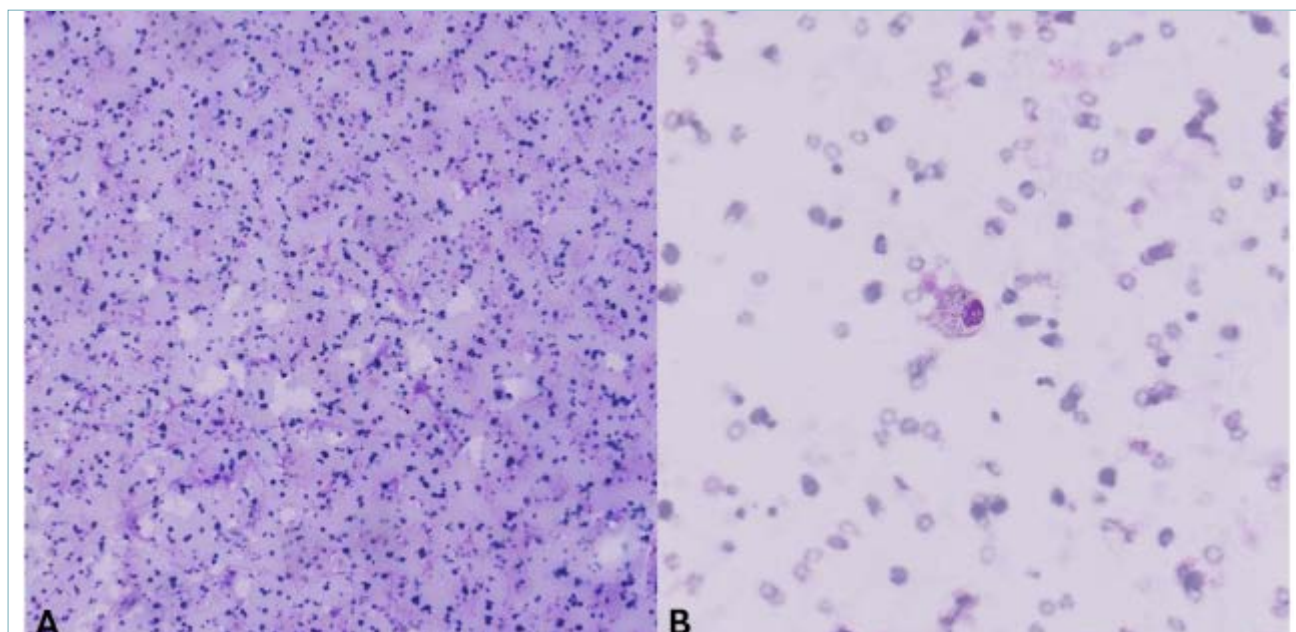


Figure 1. ID7376, ND cyst-fluid only, histological diagnosis acinic cell carcinoma. (A) low magnification showing. a fluid-proteinaceous background and histiocytes (B, 200x), without epithelial element (Diff-Quik staining).

Tab. IV).

Conversely, in scenario 2 (neoplasm benign vs malignant, Tab. V), the impact of SUMP was considered, giving its high RON and non-negligible ROM (24.4%). We considered SUMP either as a positive index for detecting malignancy, i.e., FNAs classified as SUMP were expected to be malignant (Tab. V, row 1) or as a negative index, i.e., FNAs classified as SUMP were expected to be benign neoplasms (Tab. V, row 2). Interestingly, we observed the best-case scenario when SUMP FNAs were considered as a negative index for detecting malignancy (diagnostic accuracy 93.33%).

Discussion

Our clinical experience confirms that MSRSGC is a fundamental tool for the diagnostic cytopathology of salivary gland FNAs. Indeed, we observed a high level of concordance between the ROM of our FNAs and that suggested by the NN, NB, and M categories (see Tab. I).

In particular, the NN category showed no malignancy, thus supporting our clinician's choice to follow up these patients clinically or ultrasonographically. However, 43.7% of NN were benign neoplasms on histology ($n = 7/16$, of available surgical follow-ups), though the true RON for this category is often overestimated because many of these patients may have a non-op-

erative management¹¹. Thus, the actual percentage of RON in these cases might be much lower and included in the range between the RON and the ORON percentage values (RON: 43.7%; ORON: 4.86%).

Another result that confirmed the reproducibility of the diagnostic criteria was the low ROM observed for the NB category (1.14%), lower than that recommended by both editions of MSRSGC (Tab. I). Conversely, the ROM for the M category was 94.7%, very close to the one recommended by MSRSGC, confirming that patients with an FNA diagnosed as M could be directly referred to surgery. ROSE and consequent CB preparations with ancillary studies are fundamental tools for the correct final diagnosis of M cases, highlighting the pivotal role of a correct triaging of the aspirated material¹².

Likewise, the SFM category showed a high ROM (66.7%), thereby suggesting that surgical treatment was the best option for most of these patients.

Another highly complex category to manage is the non-diagnostic (ND) category. The MSRSGC classifies salivary gland FNAs as ND when they have insufficient lesional cells, artifacts precluding proper assessment, non-mucinous cyst fluid with no epithelial component, and normal salivary gland elements in the setting of a radiologically defined mass¹³. For this category, calculating the ROM and RON is difficult because only a fraction of these cases undergoes surgical resection¹⁴. As for the NN category, several

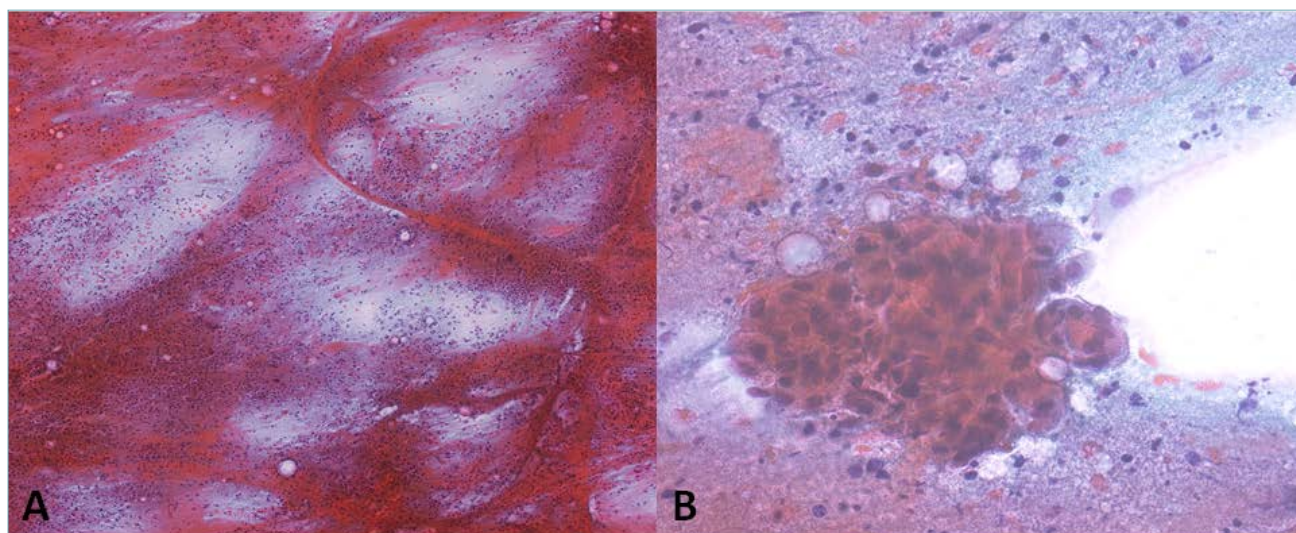


Figure 2. ID3350, AUS, histological diagnosis of Warthin tumor; (A) lympho-histiocytic background with overlapping acute inflammation (100x), and scattered groups of oncocytic cells with squamous metaplasia (B, 400x, Papanicolaou staining).

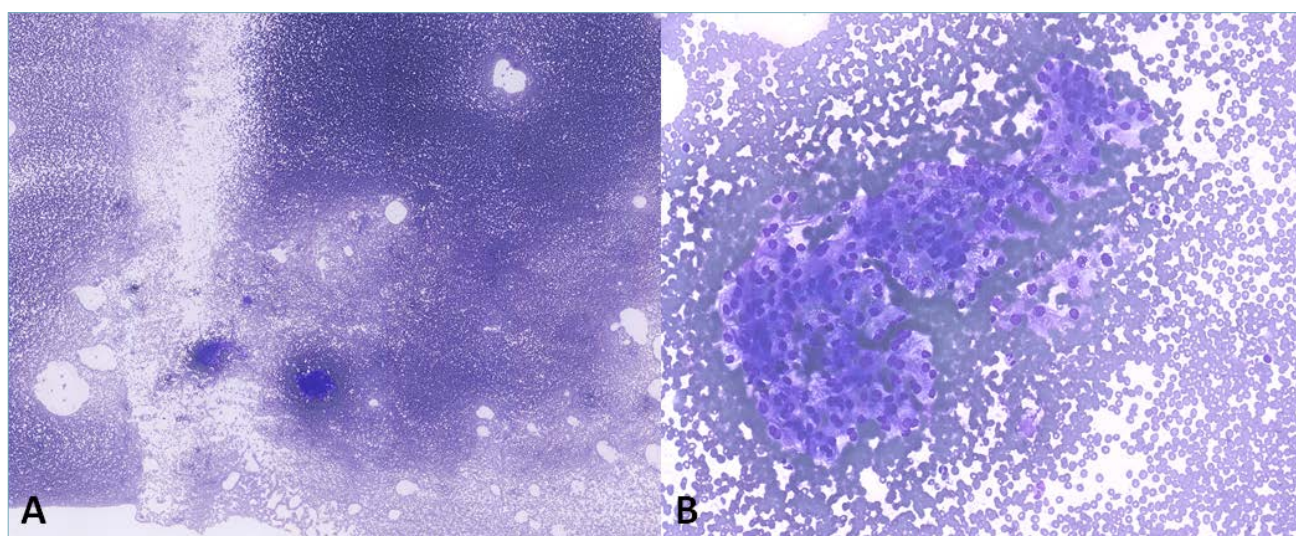


Figure 3. ID2901, SUMP, *clear cell* sub-type, histological diagnosis of acinic cell carcinoma; haematic background featuring scattered groups of neoplastic cells showing mild to moderate atypia and vacuolization of the cytoplasm (B, 400x, Diff-Quik staining).

studies have suggested that the true ROM for this category is better estimated by using a denominator that includes the overall number of cases (OROM)¹¹. The II edition of MSRSGC reports that the ROM for the ND category is 15%. In our study, instead, it was only 6%, but the OROM was even lower, namely 1.84%. To further investigate these values, we tried to have a telephone consultation with the ND patients who had

no further records at our Institution. Unfortunately, the feedback on the current health status of these patients was very scarce as a high percentage of them did not accept the consultation. However, the ones that did (18.7%) reported having recovered spontaneously or after antibiotic therapy. This suggested the reactive/inflammatory nature of the masses, and the likelihood of an improper request for FNA (data not shown).

The cytological interpretation of cystic salivary gland lesions, which account for only 8% of all salivary gland lesions¹⁵, is also very challenging. The reason is that the FNAs of these lesions are characterized by both low cellularity and a non-specific watery background, making diagnosis very difficult¹⁶. According to MSRSGC, non-mucinous cyst fluid without an epithelial component should be subcategorized as “nondiagnostic, cyst fluid only”; on the other hand, mucinous cyst fluid contents without an epithelial component should be interpreted as AUS, the reason being that FNAs with mucinous cyst content may suggest low-grade MEC¹⁷. For this reason, MSRSGC recommends first performing an US-guided FNA to drain the cyst, and, finally, targeting solid component if it is present.

In our study, 61.35% (n = 100/163) ND FNAs were categorized as “cyst-fluid only”, 35 of which had surgical follow-ups (35%). As shown in Table III, most of these lesions were non-neoplastic (n = 20, 57.14%), followed by benign neoplasms (n = 13, 37.14%), mainly WTs (69.23%). Only two cases were malignant (5.7%): one of the cases was lymphoma and the other was AcicC. The FNA smear of this latter case revealed a blood-fluid background containing numerous histiocytes filled with hemosiderin but no epithelial components (Fig. 1). The subsequent histological examination of AcicC confirmed the presence of abundant cystic and microcystic components of the lesion.

In our study, approximately 10% of salivary gland FNAs were categorized as AUS (11,1%), a percentage that closely reflects that recommended by MSRSGC (< 10%)¹⁷. The purpose of this category in MSRSGC is two-fold: to reduce the number of false-negative diagnoses in the non-neoplastic category and to reduce the number of false positive diagnoses in the neoplastic categories – mostly due to over-diagnosis of reactive and metaplastic changes, especially when associated with inflammation (Fig. 2)^{20,21}. We observed that the majority of AUS cases with histological follow-up were non-neoplastic (n = 10/33 cases on 33 total, 30.3%) and WT (n = 11/33 cases, 33.33%). Indeed, despite having a low ROM, the AUS category still suffers from a high RON, reaching up to 69.7% of cases. Accordingly, we investigated its impact on the accuracy of salivary gland FNAs in discriminating between non-neoplastic and neoplastic salivary gland lesions (Tab. IV). When we considered AUS as a positive index for detecting salivary neoplasms, we observed both higher accuracy (93.17%) and sensitivity (97%) (Tab. IV).

In an emblematic case from our series (Fig. 3), we observed a sample from a left parotid nodule from

a 62-year-old male. The sample showed a highly inflamed background and single and scattered groups of cells, suggestive of squamous differentiation. The final classification was AUS. Subsequently, the patient underwent surgical excision with a final diagnosis of WT with cystic degeneration, inflammation, and squamous metaplasia. In this regard, several studies have shown that WT is the most common benign neoplasm classified as AUS, owing to its cystic degeneration coupled with worrisome cytological features such as squamous metaplasia^{20,22,23}. In fact, in a study by Zhang et al., 72% of their WT cases demonstrated squamous metaplasia – a phenomenon most likely caused by ischemia/infarction, irradiation, or biopsy – induced tissue trauma²⁴.

Based on these clinical precedents, in our routine practice, we usually expect patients to have a neoplastic outcome after a diagnosis of AUS. In all these cases, we generally recommend further testing, including a prompt repetition of the FNA and the integration of additional imaging procedures such as MRI and CT⁴.

The purpose of adding the SUMP category in MSRSGC was to classify neoplasms in which the potential of a malignant outcome cannot be completely ruled-out. In our study, FNAs with SUMP classification were n = 57, representing approximately 6% of all FNAs. Among these, 71.93% (n = 41/57) had surgical follow-up, showing a RON of 95.1% and a ROM of 24.4%.

Considering the ROM observed for this category, we also evaluated the impact of SUMP in differentiating benign from malignant neoplasms in our clinical practice. Interestingly, the best diagnostic accuracy was obtained when SUMP was considered as a negative index for malignancy (93.33%). In fact, in our diagnostic routine, we observed a high rate of benign neoplasms on follow-ups, with PA as the most common neoplastic lesion (n = 15 out of 39 neoplastic lesions, 38.46%). In particular, PA was the most common diagnosis in the SUMP category featuring basaloid cells (n = 11, 47.8%), which represent the most frequently described subtype among SUMP diagnoses (n = 28/40, 70%, see Tab. V). Differential diagnoses may include adenoid cystic carcinoma, myoepithelioma, or other basaloid neoplasms²⁶. Conversely, in our series, the clear cell subtype, mostly represented by AcicC, showed the highest ROM (100% n = 5/5 malignancies, see Tab. V). In fact, cytoplasmic “clearing” may be associated with either benign or malignant salivary gland lesions representing non-specific changes resulting from either single or combined cellular alterations including intra-cytoplasmic lipid, mucin, glycogen, intracellular edema, and paucity of intracellular organelles (Fig. 4)⁴.

A great game changer in the complex diagnostic scenario of the SUMP category may be the adoption of molecular testing or immunocytochemical surrogate markers. Indeed, these approaches might allow for accurate diagnosis of PA. In fact, salivary gland neoplasms harbor several specific molecular alterations²⁷. Among these biomarkers are PLAG1 and HMGA2 gene fusions; both alterations have indeed been frequently associated with PA. In fact, a recent work by Freiberg et al.,²⁸ has demonstrated that NGS testing of cytological specimens can improve the classification of salivary gland neoplasms on FNA. Moreover, PLAG1 is also available as a valid surrogate immunocytochemical marker²⁹.

Conclusions

Interventional cytopathologists play an important role in salivary gland cytopathology, reinforcing the value of the MSRSGC as an essential tool in the diagnostic process. We observed a strong concordance between our findings and the risk of malignancy predicted by the MSRSGC categories, particularly for the non-neoplastic, neoplastic benign, and malignant categories. While certain challenges remain, such as the accurate interpretation of cystic lesions, our results indicate that the inclusion of the AUS and SUMP categories aids in improving diagnostic accuracy. Future studies should focus on enhancing the diagnostic process by integrating molecular testing and immunocytochemical markers to further refine the diagnostic reliability of FNAs, particularly in complex cases.

Acknowledgements

We thank Dr. Paola Merolla for editing the manuscript.

Conflict of Interest Statement

The other authors have nothing to disclose.

Funding

The authors have not declared a specific grant for this review from any funding agency in the public, commercial or not-for-profit sectors.

Authors Contribution

Conceptualization, AMC and CB; Methodology, all authors; Validation, all authors; Formal Analysis, all authors; Investigation, all authors; Resources, all authors; Data Curation, all authors; Writing – Original Draft Preparation, AMC, CB and GT; Writing – Review & Editing, all authors; Visualization, all authors; Supervision, AMC, CB and GT; Project Administration, AMC, CB and GT.

References

- 1 Rameeza A, Hemalata M. Fine-needle aspiration cytology of salivary gland lesions. *J Oral Maxillofac Pathol*. 2022; https://doi.org/10.4103/jomfp.jomfp_496_20
- 2 Viswanathan K, Patel A, Abdelsayed M, Rosado L, Soong L, Margolske E, et al. Interobserver variability between cytopathologists and cytotechnologists upon application and characterization of the indeterminate category in the Milan System for Reporting Salivary Gland Cytopathology. *Cancer Cytopathol*. 2020; <https://doi.org/10.1002/cncy.22312>
- 3 Kurtycz DFI, Rossi ED, Baloch Z, Pavelec D, Madrigal E, Vielh P, et al. Milan Interobserver Reproducibility Study (MIRST): Milan System 2018. *J Am Soc Cytopathol*. 2020; <https://doi.org/10.1016/j.jasc.2019.12.002>
- 4 Rossi ED, Baloch Z, Barkan G, Foschini MP, Kurtycz D, Pusztaszeri M, et al. Second edition of the Milan System for Reporting Salivary Gland Cytopathology: Refining the role of salivary gland FNA. *J Am Soc Cytopathol*. 2024; <https://doi.org/10.1016/j.jasc.2023.08.004>
- 5 Valstar MH, De Ridder M, Van Den Broek EC, Stuiver MM, Van Dijk BAC, Van Velthuysen MLF, et al. Salivary gland pleomorphic adenoma in the Netherlands: A nationwide observational study of primary tumor incidence, malignant transformation, recurrence, and risk factors for recurrence. *Oral Oncol*. 2017; <https://doi.org/10.1016/j.oraloncology.2017.01.004>
- 6 Rai S, Sodhi SPS, Sandhu S. Pleomorphic adenoma of submandibular gland: An uncommon occurrence. *Natl J Maxillofac Surg*. 2011; <https://doi.org/10.4103/0975-5950.85857>
- 7 Pusztaszeri M, Deschler D, Faquin Md, PhD WC. The 2021 ASCO guideline on the management of salivary gland malignancy endorses FNA biopsy and the risk stratification scheme proposed by the Milan System for Reporting Salivary Gland Cytopathology. *Cancer Cytopathol*. 2023; <https://doi.org/10.1002/cncy.22678>
- 8 Jo VY, Krane JF. Ancillary testing in salivary gland cytology: A practical guide. *Cancer Cytopathol*. 2018; <https://doi.org/10.1002/cncy.22010>
- 9 Pusztaszeri MP, Baloch ZW. Subcategorizing salivary gland neoplasm of uncertain malignant potential (SUMP) in the Milan System for Reporting Salivary Gland Cytopathology. *Cancer Cytopathol*. 2022; <https://doi.org/10.1002/cncy.22592>
- 10 Vigliar E, Carillo AM, Nacchio M, Cozzolino D, Acanfora G, Salatiello M, et al. The evolving role of interventional cytopathology from thyroid FNA to NGS: Lessons learned at Federico II University of Naples. *Cytopathology*. 9 2024; <https://doi.org/10.1111/cyt.13415>
- 11 Lui SK, Tenney T, Mullane PC, Viswanathan K, Lubin DJ. Nondiagnostic salivary gland FNAs are associated with decreased risk of malignancy compared with “all-comer” patients: Analysis of the Milan System for Reporting Salivary Gland Cytopathology with a focus on Milan I: Nondiagnostic. *Cancer Cytopathol*. 2022; <https://doi.org/10.1002/dc.25104>
- 12 Wangsiricharoen S, Lekawanvijit S, Rangdaeng S. Agreement between rapid on-site evaluation and the final cytological diagnosis of salivary gland specimens. *Cytopathology*. 2017;28(4):321–8., <https://doi.org/10.1111/cyt.12428>
- 13 Johnson DN, Antic T, Reeves W, Mueller J, Lastra RR, Cipriani NA, et al. Histopathologic and clinical outcomes of Milan System categories “non-diagnostic” and “non-neoplastic” of salivary gland fine needle aspirations. *J Am Soc Cytopathol*. 2021; https://doi.org/10.4103/ijpm.ijpm_676_21
- 14 Hirata Y, Higuchi K, Tamashiro K, Koja K, Yasutomi Y, Matsuzaki A, et al. Application of the Milan System for Reporting Salivary Gland Cytopathology: A 10-Year Experience in a Single Japanese Institution. *Acta Cytol*. 2021; <https://doi.org/10.1159/000510990>

- ¹⁵ Pantanowitz L, Thompson LDR, Rossi ED. Diagnostic Approach to Fine Needle Aspirations of Cystic Lesions of the Salivary Gland. *Head Neck Pathol.* 2018; <https://doi.org/10.1007/s12105-018-0904-8>
- ¹⁶ Maleki Z, Allison DB, Butcher M, Kawamoto S, Eisele DW, Pantanowitz L. Application of the Milan System for Reporting Salivary Gland Cytopathology to cystic salivary gland lesions. *Cancer Cytopathol.* 2021; <https://doi.org/10.1002/cncy.22363>
- ¹⁷ Rossi ED, Faquin WC. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC): An international effort toward improved patient care—when the roots might be inspired by Leonardo da Vinci. *Cancer Cytopathol.* 2018; <https://doi.org/10.1002/cncy.22040>
- ¹⁸ Xu M, Deng L, Peng K, Wei X, Xie M, Liu M, et al. Rapid on-site evaluation improves diagnostic performance of fine-needle aspiration cytology for salivary lesions: Comparison of data from two cancer centers in southern China. *Diagn Cytopathol.* 2024; <https://doi.org/10.1002/dc.25277>
- ¹⁹ Bellevicine C, Vigliar E, Malapelle U, Pisapia P, Conzo G, Biondi B, et al. Cytopathologists can reliably perform ultrasound-guided thyroid fine needle aspiration: a 1-year audit on 3715 consecutive cases. *Cytopathology.* 2016; 10.1111/cyt.12243
- ²⁰ Wangsiricharoen S, Maleki Z. Risk stratification and clinical outcome in the atypia of undetermined significance category in the Milan System for Reporting Salivary Gland Cytopathology. *Cancer Cytopathol.* 2021; 10.1002/cncy.22352
- ²¹ Lubin D, Buonocore D, Wei X, Cohen J, Lin O. The Milan System at Memorial Sloan Kettering: Utility of the categorization system for in-house salivary gland fine-needle aspiration cytology at a comprehensive cancer center. *Diagn Cytopathol.* 2020; <https://doi.org/10.1002/dc.24350>
- ²² Cormier CM, Agarwal S. Utility of the Milan System for Reporting Salivary Gland Cytology, with focus on the incidence and histologic correlates of atypia of undetermined significance (AUS) and salivary gland neoplasm of uncertain malignant potential (SUMP): A 3-year institutional experience. *Cancer Cytopathol.* 2022; <https://doi.org/10.1002/cncy.22538>
- ²³ Torous VF, Faquin WC. The Milan System classification of Warthin tumor: A large institutional study of 124 cases highlighting cytologic features that limit definitive interpretation. *Cancer Cytopathol.* 2022; <https://doi.org/10.1002/cncy.22588>
- ²⁴ Zhang X, Baloch ZW, Cooper K, Zhang PJ, Puthiyaveetil R, LiVolsi VA. The significance of mucinous metaplasia in Warthin tumor: a frequent occurrence and potential pitfall. *Hum Pathol.* 2020; <https://doi.org/10.1016/j.humpath.2020.03.008>
- ²⁵ Israel A, Griffith CC. Application of the Milan system for reporting salivary gland cytopathology to core needle biopsies of the parotid gland. *Histopathology.* 2024; <https://doi.org/10.1111/his.15200>
- ²⁶ Allison DB, Smith AP, An D, Miller JA, Shafique K, Song S, et al. Assessing the diagnostic accuracy for pleomorphic adenoma and Warthin tumor by employing the Milan System for Reporting Salivary Gland Cytopathology: An international, multi-institutional study. *Cancer Cytopathol.* 2021; <https://doi.org/10.1002/cncy.22339>
- ²⁷ Carillo AM, De Luca C, Pisapia P, Vigliar E, Ikenberg K, Freiburger SN, et al. Molecular testing in salivary gland cytopathology: A practical overview in conjunction with the Milan system. *Cytopathology.* 2024; <https://doi.org/10.1111/cyt.13363>
- ²⁸ Freiburger SN, Ikenberg K, Van Egmond D, Claerhout S, Van Wezel T, Bempt IV, et al. Molecular analysis using SalvGlandDx improves risk of malignancy estimation and diagnosis of salivary gland cytopathology: An exploratory multicenter study. *Cancer Cytopathol.* 2024; doi: 10.1002/cncy.22814
- ²⁹ Rotellini M, Palomba A, Baroni G, Franchi A. Diagnostic Utility of PLAG1 Immunohistochemical Determination in Salivary Gland Tumors. *Appl Immunohistochem Mol Morphol.* 2014; <https://doi.org/10.1097/PAI.0b013e3182936ea7>