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Journal homepage: https://www.achr.co.in/

Review Article

The indeterminate categories of the Milan system for reporting salivary gland cytopathology (MRSGC): A minireview

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ARTICLE INFO

Article history:
Received 01-03-2024
Accepted 27-03-2024
Available online 04-05-2024

Keywords:
Salivary gland
FNAC
Milan System for Reporting Salivary Gland Cytopathology

ABSTRACT

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC), first published in 2018, attempted to develop a standardized and objective system of reporting salivary gland fine needle aspiration smears. It was widely accepted by the cytopathologists who started using it in their day-to-day practice. With the publication of new literature, the Milan system was updated and the 2^{nd} edition was released in 2023. The 5^{th} WHO classification of salivary neoplasms, ancillary tests, and imaging characteristics of various salivary neoplasms have also been highlighted in the new edition. The 3 indeterminate categories of the Milan system of reporting are the atypia of undetermined significance (AUS), salivary gland tumor of undetermined malignant potential (SUMP), and suspicious for malignancy (SM) that create major dilemmas for the reporting cytopathologists. The present minireview highlights the criteria of diagnosis and further workup of the various lesions in these categories.

1. Introduction

Salivary gland lesions represent a widely heterogeneous group of lesions ranging from non-neoplastic conditions to high-grade malignant tumors. This is complicated by overlapping morphology and variations within each tumor, sometimes making diagnosis difficult. Fine needle aspiration cytology (FNAC) is an established first-line diagnostic modality for salivary gland lesions. It is popular with clinicians and patients because it is less expensive, less invasive, and has a relatively high diagnostic accuracy and short turnaround time (TAT).

The various studies have shown an overall sensitivity and specificity of salivary gland FNA ranging from 91 to 93.94% and 94.92 to 97.48%, respectively.[1–3] However, when it comes to specifically subtype a tumor, it has a wide range from 48 to 94%.[1–3] Ultrasound (US) -guided FNAC may be helpful in cases where the lesion is deep-seated or cystic. The ultrasound also helps to localize the lesion to be arising from the salivary gland. Various other imaging modalities like computed tomography (CT), magnetic resonance imaging (MRI), as well as nuclear and molecular imaging techniques, have also been added to the armamentarium of diagnostic tools.[4]

In 2018, the first edition of The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was released, the editors being William C. Faquin and Esther Diana Rossi. Based on the large data from the published articles since the release of the first edition, the MSRSGC was updated and the second edition was released in 2023. The primary objective of MSRSGC was to develop a standardized and objective system of reporting salivary gland FNAs in the form of reproducible diagnostic categories.

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https://doi.org/10.18231/j.achr.2024.003

2. Discussion

MSRSGC recommends reporting of salivary gland cytology smears by 6 diagnostic categories. These categories are linked to risk of malignancy (ROM) which are calculated based on the published literature available at that time and also offer clinical management guidelines. (Table 1). Out of these diagnostic categories, the Atypia of undetermined significance (AUS), the Salivary gland tumor of undetermined malignant potential (SUMP), and Suspicious of malignancy are the 3 indeterminate categories where a definite diagnosis cannot be rendered on cytology and will be discussed in this review.

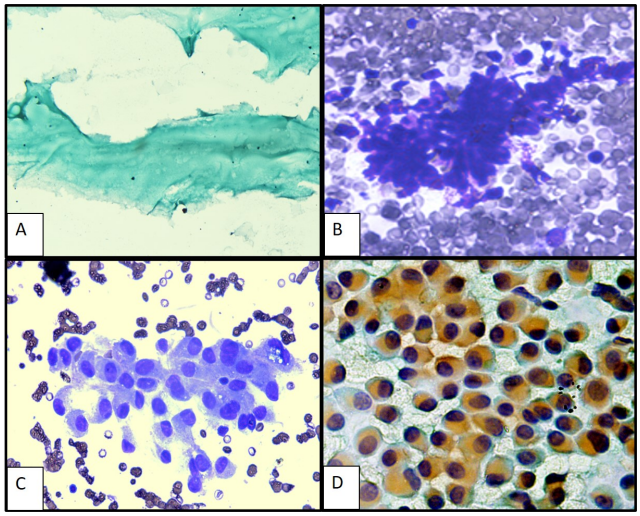

Figure 1: A: AUS: Mucinous material only without epithelial cells from an aspirate of left parotid gland (PAP stain X 200 magnification); **B**: SUMP: Tight cluster of basaloid cells with scant cytoplasm and no matrix in a hemorrhagic background (MGG stain X 400 magnification); **C:** SUMP: Loose groups of cells with oncocytic cytoplasm suggestive of cellular oncocytic neoplasm (MGG stain X 400 magnification); **D:** SUMP: Aspirate from a PA showing only myoepithelial cells without any matrix (PAP stain X 400 magnification)

3. Atypia of Undetermined Significance (AUS)

The FNA is done to arrive at a rapid and accurate diagnosis of salivary gland lesions particularly to differentiate between the nonneoplastic and neoplastic lesions. However, the sample obtained or the smears prepared are sometimes not satisfactory enough to make this distinction. In this subset of AUS, all the problematic FNAs are included in which the cells are qualitatively or quantitatively insufficient to classify the aspirate into either neoplastic or non-neoplastic groups. The causes of these can be classified as.[4,8]

1. Technical (preanalytical) factors: poor FNA technique, poor sampling, poor slide preparation, air drying artifacts, excessive clotting artifacts, obscuring substances like blood, poor staining, etc.
2. Inherent characteristics of the lesion - cystic degeneration, mucin, fibrosis, necrosis, lymphoid lesion, etc.

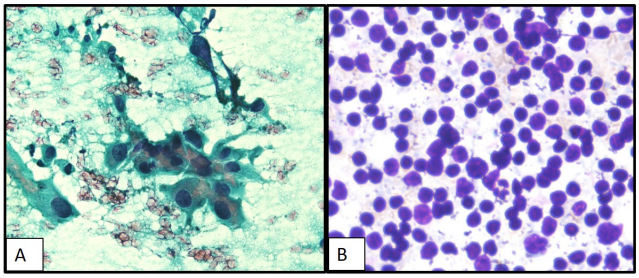

Figure 2: A: Suspicious for high-grade carcinoma (SM): Markedly atypical cells in an otherwise paucicellular smear (PAP stain X 400 magnification); **B**: Suspicious for lymphoma (SM): Smear shows the presence of a mixed pattern with a predominance of intermediate-sized lymphocytes. Ancillary studies are needed for further classification (MGG stain X 400 magnification)

These causes result in the inability to categorize a lesion into either neoplastic or nonneoplastic category. In general, the idea is that the AUS favors a benign process but cannot rule out the possibility of a neoplasm due to the lack of adequate cytomorphological features of a neoplasm.[4,8] This category reduces the false positive diagnosis of the neoplastic category and the false negative diagnosis of the nonneoplastic category.[4]

As per the MSRSGC 2^{nd} edition, the diagnostic criteria under this category are:[4]

1. Reactive and reparative atypia which are indeterminate for neoplasm.
2. Metaplastic changes like squamous, oncocytic metaplasia, etc. indeterminate for neoplasm.
3. Low cellularity specimens with rare atypical cells that are suggestive, but not diagnostic, of a neoplasm.
4. Specimens with preparation artifacts in which neoplasm cannot be ruled out.
5. Mucinous cystic lesions with scant/absent epithelial cells.
6. Atypical lymphoid infiltrates, where a lymphoproliferative disorder cannot be ruled out based on morphology but at the same time are not atypical as to include it under the suspicious for malignancy category and ancillary tests like flow cytometry is necessary for diagnosis.

An aspirate that shows cyst fluid with cyst macrophages is reported as non-diagnostic, cyst fluid only. However, if mucinous material is obtained with scant/absent epithelial cells, it is categorized as AUS, as per MSRSGC. This is because a mucinous material can be obtained from benign

Table 1: Diagnostic categories of salivary gland lesions with risk of malignancy and management guidelines as perMSRSGC[4]

S.No.	Diagnostic categories	ROM (1^{st} ed)	ROM (2^{nd} ed)	Management
I	Non-diagnostic	25%	15%	Clinical and radiologic correlation/repeat FNA
II	Non-neoplastic	10%	11%	Clinical follow-up and radiologic correlation
III	**Atypia of undetermined significance (AUS)**	20%	30%	Repeat FNAC or surgery
IV	Neoplasm			
IVA	Benign	<5%	<3%	Surgery or clinical follow-up
IVB	**Salivary gland tumor of undetermined malignant potential (SUMP)**	35%	35%	Surgery [Intraoperative consultation (frozen section) to determine the extent of surgery]
V	**Suspicious of malignancy**	60%	83%	Surgery [Intraoperative consultation (frozen section) to determine the extent of surgery]
VI	Malignant	90%	98%	Surgery (type and grade of malignancy determine the extent of surgery)

The indeterminate categories are highlighted in **bold**.

Table 2: Molecular alterations in salivary gland neoplasms[5–7]

Histologic types	Gene rearrangements
Pleomorphic adenoma	PLAG1 gene rearrangement >> HMGA 2 gene rearrangement
Mucoepidermoid carcinoma	CRTC1::MAML2 fusion >>CRTC3::MAML2 fusion
Adenoid cystic carcinoma	MYB::NFIB >>MYBL1::NFIB> NOTCH1 mutation
Acinic cell carcinoma	NR4A3 gene upregulation>> HTN3::MSANTD3 fusion
Secretory carcinoma	ETV6::NTRK3 fusion >>ETV6::RET fusion
Basal cell adenoma	CTNNB1 mutation
Basal cell adenocarcinoma	PIK3CA mutation
Salivary duct carcinoma	HRAS mutations, TP53 mutations, PIK3CA mutations, BRAF mutations, PTEN deletions, AR gene activation, and HER2 (ERBB2) amplification; PLAG1 and HMGA 2 gene rearrangement (in carcinoma ex pleomorphic cases of SDC)
Hyalinizing clear cell carcinoma	EWSR1::ATF1 gene fusion
Epithelial-myoepithelial carcinoma	PLAG1 and HMGA 2 gene rearrangement> HRAS mutation
Polymorphous Adenocarcinoma	Hot spot point E710D mutations in the PRKD1 gene
Canalicular adenoma of minor salivary glands	PRKD1-3 translocations, ARID1A and DDX3X are partner genes
Mucinous adenocarcinoma	AKT1::E17K ; TP53 alterations
Intraductal catcinoma	RET fusions; BRAF V600E mutations; HRAS, PIK3CA, and TP53 mutations
Microsecretory adenocarcinoma	MEF2C::SS18 fusions

conditions like mucocele or mucus retention cyst as well as a malignant lesion like low-grade mucoepidermoid carcinoma.[4] To complicate matters, in the latter, an aspirate can yield mucous cells that may mimic histiocytes in a mucus background.[8] Mucinous material can also be obtained from other neoplastic conditions like Warthin tumor (WT), pleomorphic adenoma (PA) with mucinous metaplasia, papillary cystadenoma/cystadenocarcinoma, etc.[4] With such varied diagnoses ranging from benign lesions to malignant lesions, a diagnosis of AUS is best suited for mucinous fluids. (Figure 1A)

However, it is important to note that AUS should not be made a waste basket and every possible attempt should be made to classify them into a more specific category. For quality control, the MSRSGC recommends that AUS should be < 10% of all the diagnoses rendered for salivary cytology.[4]

Since this is a heterogeneous group with many problematic cases, the ROM for this category is estimated at 30%.[4,8] The decision on how to manage the AUS cases can be taken by a multidisciplinary team comprised of clinicians, radiologists, and pathologists. A cyst that resolves completely on aspiration may be followed up while if a residual mass is there after evacuation, it should be sampled under US guidance.[4] Similarly, since about one-third of cystic salivary gland lesions are neoplastic, surgical resection can be done in cysts that do not resolve on aspiration or in cysts that recur.[4]

AUS also includes aspirates that yield atypical lymphoid proliferations where a lymphoproliferative disorder

cannot be excluded based on cytomorphology. These cases should be properly evaluated by flow cytometry, immunocytochemistry, immunohistochemistry, and/or histopathological examination of tissue biopsy.[4,8]

Thus, it should be emphasized that all cases of AUS should have a proper clinicoradiological correlation and may need ancillary tests to determine the preferred line of management.

The use of rapid on-site evaluation (ROSE) of smears, proper FNAC technique, processing, and use of ancillary tests will help to reduce the number of AUS cases.

4. Salivary Gland Tumor of Undetermined Malignant Potential (SUMP)

It is a subcategory (Category IVB) under the Neoplasm category (Category IV), the other subcategory being benign neoplasm (Category IVA). (Table 1)

The SUMP category includes cases in which the cytomorphologic features are diagnostic of a neoplasm but it cannot be stamped as benign or malignant category with certainty.[8,9]

The following are the common scenarios where it is prudent to give a diagnosis of SUMP although the list is not all-inclusive:

1. Cellular basaloid neoplasms
2. Cellular oncocytic /oncocytoid neoplasms
3. Cellular Neoplasm with Clear Cell Features
4. Cellular neoplasm with mixed features
5. Benign neoplasms with atypical features

The cellular basaloid neoplasms are composed of cohesive sheets of basaloid cells with scant cytoplasm and a high N:C ratio. (Figure 1B) Based on the presence of different types of matrix or hyaline stroma, the MSRSGC 2^{nd} edition has further subclassified this group into:[9]

1. Cellular basaloid neoplasm with scant fibrillary matrix e.g cellular pleomorphic adenoma, basal cell adenoma/adenocarcinoma, epithelial-myoepithelial carcinoma (EMC), carcinoma ex pleomorphic adenoma, etc.
2. Cellular basaloid neoplasm with hyaline stroma e.g basal cell adenoma/adenocarcinoma, adenoid cystic carcinoma (Ad CC), epithelial-myoepithelial carcinoma, carcinoma ex pleomorphic adenoma, etc.
3. Cellular basaloid neoplasm with mixed/other matrix: cellular pleomorphic adenoma, polymorphous adenocarcinoma, adenoid cystic carcinoma, carcinoma ex pleomorphic adenoma, etc.
4. Cellular basaloid neoplasm with minimal matrix to no matrix: cellular pleomorphic adenoma, myoepithelioma, canalicular adenoma, adenoid cystic carcinoma, myoepithelial carcinoma, carcinoma ex pleomorphic adenoma, etc.

Thus, it is evident that a particular morphology has several differentials, from benign neoplasm to malignant neoplasm, which are always not possible to diagnose with certainty.

Similar to the cellular basaloid neoplasms, cellular oncocytic/oncocytoid (oncocyte-like) neoplasms are best categorized under this SUMP category. These are neoplasms in which the predominant pattern is of cells having a moderate amount of granular, oncocytic cytoplasm with a variable background. (Figure 1C)

This is because the presence of oncocytes can be seen in various conditions starting from nodular oncocytosis to benign tumors like oncocytoma, WT, sclerosing polycystic adenoma, and even oncocytic variants of low-grade tumors like mucoepidermoid carcinoma (MEC), acinic cell carcinoma (ACC) and secretory carcinoma (SC).[9] Thus, the diagnosis of oncocytoma into the category of benign neoplasm is to be avoided when material for ancillary studies is not available or good clinical and radiologic data is not available. They are better categorized as SUMP.[9]

The evaluation of background in oncocytic neoplasms is important as they provide valuable clues to the diagnosis e.g. the prominent lymphoid background of WT, mucinous background in oncocytic mucoepidermoid carcinoma (MEC), stripped nuclei (more in smear preparations), and sometimes lymphocytes in acinic cell carcinoma (ACC), etc.

The 3^{rd} category of tumors under SUMP are cellular neoplasms with clear cell features i.e neoplasms that are composed predominantly of cells with a moderate amount of cytoplasm which is clear or vacuolated or granular or foamy or any combination of these, but not meeting the criteria of oncocyte.[9] High-grade nuclear features like atypical mitosis, nuclear pleomorphism, high mitotic activity, necrosis, etc are generally absent as this subgroup usually comprises benign tumors like myoepithelioma or low-grade carcinomas like ACC, low-grade MEC, hyalinizing clear cell carcinoma (HCCC), myoepithelial carcinoma, EMC, etc.[9]

Cellular neoplasms with a mixture of the above features like basaloid, oncocytic, clear cell morphology is also included in this category.

In addition, benign tumors with atypical features may also be included in this category of SUMP. For example, cellular PA rich in myoepithelial cells with scant to absent matrix (Figure 1D), PA with myoepithelial cells which are spindled or clear cell morphology, PA with mucinous background and /or squamous/mucinous metaplasia, PA with scattered atypical cells, etc may raise the possibility of several differentials. Such features, if present, warrant a diagnosis of SUMP rather than a benign diagnosis. Similarly, infarction within a WT may give rise to necrotic debris and atypical squamous cells mimicking a squamous cell carcinoma.[10] Again, metaplastic squamous cells in a mucinous background in WT can look like a low-grade MEC.[10] Such dubious lesions occurring in a benign tumor

are also included in the SUMP category.

As expected, the ROM of this category is as high as 30% in both the 1^{st} and 2^{nd} editions of MSRSGC as this category includes many low-grade carcinomas in addition to benign neoplasms.

The number of SUMP cases may be reduced and a definite benign or malignant category may be assigned by the use of several ancillary techniques that are available now. The major ancillary techniques that are available and can be integrated into the diagnostic workflow are as follows:

1. Histochemistry or use of special stains
2. Immunohistochemistry/immunocytochemistry
3. FISH
4. RT-PCR
5. NGS
6. Flow Cytometry

However, most of the centers lack advanced cytogenetics or molecular techniques, or even immunochemistry, making the use of ancillary techniques inaccessible in most cases.

5. Histochemistry

PAS and PAS with diastase (d-PAS) can be used to highlight the zymogen granules in ACC, glycogen in HCCC, and intracytoplasmic mucin in MEC and SC.[5]

Neutral and acid mucin can be demonstrated by mucicarmine stain and Alcian blue (pH 2.5) respectively while lipid droplets indicating sebaceous differentiation can be highlighted by fat stains Oil Red O and Sudan IV on unfixed cells.[5]

6. Immunochemistry (IC)

Immunochemistry can be done on cytology smears (immunocytochemistry) or sections prepared from cell blocks (immunohistochemistry). The latter is easier to perform and standardize though both yield good results if done meticulously. The IC panel is chosen as per the cytological findings on the smears.

6.1. For example

IC panel for basaloid neoplasms [which include PA, AdCC, basal cell adenoma (BCA), Basal cell adenocarcinoma (BCAdc), PAC, myoepithelioma/myoepithelial carcinoma, and EMC] should include the following markers: at least 2 of the basal markers (p40, p63, CK 5/6, S100, SMA, Calponin), CAM 5.2 or EMA, Ki 67, PLAG1, HMGA2, MYB, CD117, beta catenin, and/or LEF1.

Morphologically, most of these tumors produce variable amounts of matrix. In 50% to 60% of all PA, there is a rearrangement of the PLAG1 gene (encoded on 8q12) while 10%-20% of cases of PA harbor rearrangements of the HMGA2 gene (encoded on 12q14-15).[11–14] Immunochemical stains are available to detect the overexpression of PLAG1 and HMGA2 in PA. PLAG1 is a more sensitive immunostain being positive for 70-95% of cases of PA.[15–17] HMGA2 is more specific (approximately 96%) and less sensitive (approximately 30%)[18] A recent study by Matsuyama et al showed that a combination of PLAG1 and HMGA2 immunohistochemistry could detect approximately 85% of all cases of PA.[17] However, cases of carcinoma ex pleomorphic adenoma also show positivity for these markers so these markers cannot distinguish benign from malignant.[5] They are also positive in SDC that arise as carcinoma ex pleomorphic adenoma. However, those cases are also positive for androgen receptors (AR) which is characteristic of SDC.

Myoepithelioma/ myoepithelial carcinomas show positivity for all the myoepithelial markers (p40, p63, SOX10, S-100, SMA, calponin) and variable expression of PLAG1 and HMGA2 but the distinction from benign to malignant can only be done on histology by seeing the status of the invasion. The IC profile of EMC is also similar to Myoepithelioma/ myoepithelial carcinomas. Additionally, MYB may be positive in a subset of cases of EMC.[5] Most of the cases of Ad CC show strong nuclear positivity for MYB and membranous positivity for CD117 (c-kit).[5] Although CD117 can also be positive in BCA/BCAdc and PAC, unlike Ad CC, MYB is negative or only focally positive in these tumors. Nuclear stain for β-catenin and/or LEF 1 can help in the detection of BCA/BCAdc but the basal cell adenoma and carcinoma cannot be distinguished on FNA as this requires the demonstration of tumor invasion on histopathology.[5] However, intraoperative frozen sections may be helpful in these cases and guide the extent of surgery.

The IC panel for oncocytic/oncocytoid neoplasms (which includes Oncocytoma, WT, ACC, SC, MEC, and salivary duct carcinoma) should include myoepithelial markers (p63, p40, S100, SOX10), DOG1, GATA 3, Mammoglobin, AR, mucicarmine, etc.

Oncocytoma/WT and MEC both show positivity for p40 and p63 but their distribution is diffuse in MEC while it is limited to a single basal layer of cells in Oncocytoma/WT.[5] Again mucicarmine will be positive in MEC. All the other oncocytic/oncocytoid neoplasms are negative for p40 and p63. A combination of nuclear positivity for SOX 10 and a membranous and canalicular pattern of DOG1 helps in the diagnosis of ACC. IC for NR4A3 is a surrogate marker for NR4A3 translocation which is present in most ACC.[5] Nuclear staining for NR4A3 has shown high sensitivity and specificity in cytologic material.[19]

GATA 3, Mammoglobin, and S 100 help diagnose SC cases a subset of which show oncocytic cells. Additionally, membranous immunostaining for Pan-TRK helps diagnose

TRK fusion-positive SC, however, the data is limited.[5] MUC4 immunostain is also positive in SC unlike PAC, ACC, and SDC but it is also frequently diffusely positive in all cell types of MEC. GATA 3 and Androgen receptor (AR) is positive in SDC which often helps to clinch the diagnosis of SDC.[5]

The IC panel for neoplasms with clear cell features (which include ACC, low-grade MEC, HCCC, myoepithelial carcinoma, and EMC) includes the myoepithelial markers (p63, p40, S100, SOX10) and DOG1. The immune profiles of all these tumors except CCC have already been discussed. In CCC we have positivity for p40 and p63 while SOX 10 and S100 are negative.[5]

Though the cases of high-grade carcinomas are rarely categorized as SUMP, it will be nice to discuss the IC panel of these tumors as well as most of them have already been elucidated. The high-grade carcinomas include high-grade MEC, SDC, primary and metastatic squamous cell carcinomas, lymphoepithelial carcinomas, poorly differentiated carcinomas, neuroendocrine carcinomas (NE), and metastatic cancers. As there is a wide range of tumors, the IC panel is larger but guided by morphology. The markers included are the myoepithelial markers, mucin, CK 5/6, CK 8/18, AR, GATA 3, neuroendocrine markers (chromogranin, synaptophysin, CD56), CK 20, and site-specific IC for metastatic tumors based on morphology and/or prior history (TTF-1 for thyroid/lung primary, CDX2 and SATB2 for enteric primary, PAX-8 for renal primary, etc)

MEC is positive for p40, p63, mucin, CK5/6, and focal CK 8/18 while SDC is positive for AR, GATA 3, and CK 8/18. Primary and metastatic squamous cell carcinomas and lymphoepithelial carcinomas are positive for p40, p63, CK 5/6, and variable positivity for CK8/18. NE are positive for the NE markers while metastatic cancers show an immune profile of the primary site.[5]

Many salivary gland tumors have characteristic molecular alterations. (Table 2) They can be highlighted by molecular and cytogenetic methods like FISH, PCR, and NGS. However, a detailed discussion of these procedures is beyond the scope of this article.

As discussed, PA and carcinoma ex PA (irrespective of morphology) harbor PLAG1 and HMGA2 rearrangements. The most characteristic molecular abnormality in MEC is MAML2 rearrangement.[5] Most commonly there is CRTC1::MAML2 fusion which is due to the translocation t(11;19)(q21;p13) and rarely a CRTC3::MAML2 fusion secondary to translocation t(11;15)(q21;q26) can be seen.[8,18,19,20] AdCC is characterized by the translocation t(6;9)(q21–24;p13–23) which involves MYB and NFIB genes in about half of all cases.[6,20,21] While MYB overexpression is detected in up to 90% of all AdCC, activating mutations of NOTCH1 are seen in approximately 10% of cases of AdCC.[6] NOTCH mutation is mostly associated with the solid type of AdCC and poorer prognosis.[6]

Translocation t(12;15)(p13;q25) resulting in ETV6::NTRK3 fusion is the hallmark of SC.[6,20,21] The translocation t(4;9)(q13;q31) results in constitutive upregulation of NR4A3 (encoded on 4q13) through enhancer hijacking in cases of ACC.[5,6,19]

30–80% of BCA cases show CTNNB1 mutations, while BCAdc shows activating mutations in PIK3CA, usually without CTNNB1 mutation but showing β catenin expression none the less.[22] SDC harbors HRAS mutations, TP53 mutations, PIK3CA mutations, BRAF mutations, PTEN deletions, AR gene activation, and HER2 (ERBB2) amplification in up to 35% of cases.[5,6]HER2 amplification when present can be treated with Trastuzumab.

EWSR1 rearrangements are seen in HCCC and Myoepithelial carcinoma, clear cell variant.[23]

7. Fluorescent in Situ Hybridization (FISH)

All the mutations are nicely picked up by FISH or NGS. For FISH, cytologic material (smears, cytospin material) may be preferable to FFPE cell blocks as they are free from truncation artifacts due to routine tissue processing. However, cell blocks also give good results. When specific translocations are known break-apart FISH probes are designed to flank on either side of a gene of interest. In the presence of a translocation, the two colors will lead to a split signal favoring the diagnosis.[5] FISH is a sensitive test when performed properly and can help in diagnosis with a limited number of lesional cells.[5] Though a bit expensive, it is quite popular among pathologists for its ease and accuracy.

Polymerase chain reaction (PCR) is a more sensitive test than FISH for the detection of known translocations and can give excellent results on cytological preparations even with 50-100 lesional cells.[5] However, unlike FISH it cannot detect unknown molecular variants.[5]

Next-generation sequencing (NGS) is a relatively new high-throughput, highly sensitive molecular technology platform with the advantage that a large number of genes can be analyzed simultaneously and that too of multiple patients in a single run.[5,24] It is a rapidly evolving field and studies have shown its effectiveness in salivary gland tumor cytology.[24,25] However, the major limitations are the high cost and requirement of robust bioinformatics to handle the analysis and interpretation of large amounts of data.[26]

SalvGlandDx is an all-in-one RNA-based NGS panel that detects mutations, fusions, and gene expression levels of 27 genes (including NR4A3) found in salivary gland tumors.[27]

Flow Cytometry (FC) is used to diagnose atypical lymphoproliferative lesions by immunophenotyping. Many atypical lymphoid proliferations are designated the AUS category or SM category depending upon their quantitative

and qualitative attributes, where a definite diagnosis of lymphoma cannot be rendered on cytology alone. Such lesions are best evaluated by FC. ROSE improves adequacy and helps in triaging the lesions. Atypical or suspicious lymphoid cells on ROSE should prompt dedicated pass(es) from the lesion and the aspirates and needle rinses should be submitted for FC. The sample can be sent on Roswell Park Memorial Institute (RPMI) medium or even sterile saline if it is processed within a short time, i.e. within the same day.[5] Stacchini et al. showed that FC can very efficiently differentiate between reactive conditions and lymphomas, particularly in problematic low-grade lymphomas like low-grade B-cell and mucosa-associated lymphoid tissue (MALT).[28] They also reported a sensitivity of 100% and a specificity of 83% in diagnosing and classifying lymphoproliferative disorders of salivary glands by combining cytology with FC.[5,28]

The role of using the ancillary tests to categorize the cases of AUS, SUMP, and SM into benign and malignant categories cannot be undermined. However, in most of the centers, these facilities are either not available or are too costly for patients to afford thus limiting their true potential.

The appropriate management of SUMP is best decided in a multidisciplinary tumor board which includes a team of oncologists (medical oncologists, oncosurgeons, radiation oncologists, etc), radiologists, and pathologists. A preoperative MRI or CT will be helpful to evaluate the extent of the tumor and assess the neck region.[5] A repeat FNAC or core needle/open biopsy may be performed if indicated. In cases where there is a need for surgical excision, a nerve-sparing surgical resection is done in most cases. The intraoperative frozen section is helpful to comment on tissue diagnosis, margin status and help to decide whether neck dissection is indicated or not.

8. Suspicious for Malignancy (SM)

This indeterminate category includes cases that are suggestive of malignancy but the cytological criteria are qualitatively or quantitatively short of a definite diagnosis of malignancy.

Thus, it is reserved for cases in which the degree of atypia is higher than AUS or SUMP and some but not all features of a specific malignant neoplasm are present.[8,29]

The purpose of having this category is to maintain a high positive predictive value (PPV) for the malignant category almost approaching 100%.[8] The ROM in this SM category is also high and is reported to be 83% in the 2^{nd} edition of MSRSGC.[29]

However, it is not enough to give a diagnosis as SM but it is recommended that it should further be subclassified as suspicious for a primary salivary gland malignancy, metastasis, or lymphoma.[8,29] The majority of the cases of SM are suboptimal samples of high-grade salivary gland malignancies.[29,30]The conditions where this SM diagnosis may be rendered include:[8,29]

1. Highly atypical cells but are obscured by inflammation or blood or interpretation is hindered by poor smear preparation, artifacts, or poor cell preservation
2. Paucicellular smears with cytologic features not enough to specify a particular malignant neoplasm (eg. AdCC, ACC, etc)
3. Markedly atypical cells and/or cytologic features suspicious of malignancy in a sparsely cellular smear (Figure 2A)
4. Paucicellular samples with atypical features suggestive of lymphoma (Figure 2B), neuroendocrine neoplasm, or metastatic tumors

A subset of cases of SM may be, with the help of ancillary techniques like IC, FISH, or FC, categorized into the malignant category.

This category, however, is not equivalent to the malignant category and hence radical surgery (including facial nerve sacrifice), chemotherapy, or radiotherapy cannot be instituted based on this diagnosis alone.[8,29] The appropriate management of SM is best decided in a multidisciplinary tumor board. Depending on the clinicoradiological correlation and cytological findings, repeat FNA, biopsy (Core needle/open biopsy) or surgical excision may be considered.[29] The intraoperative frozen section may prove beneficial in the cases of surgical resection to guide the extent and course of surgery.[8]

9. Conclusion

The 3 indeterminate categories of MSRSGC are the grey areas of salivary gland cytology. The use of good practices of sample collection and processing, taking radiological guidance whenever necessary, and routine use of ROSE will help to reduce the number of cases in these categories. Judicious use of ancillary techniques, wherever available, will be helpful in a subset of cases. Clinicoradiological correlation and consultation in a multidisciplinary tumor board are necessary for optimal clinical management in these cases.

10. Source of Funding

None.

11. Conflict of Interest

None.

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Cite this article: Chakrabarti I, Mazumder S. The indeterminate categories of the Milan system for reporting salivary gland cytopathology (MRSGC): A minireview. *IP Arch Cytol Histopathology Res* 2024;9(1):9-16.