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REVIEW

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Updates in head and neck cytopathology: Insights from European Congress of Pathology Short Course

Ivana Kholová[1,2] | Ashish Chandra[3] | William C. Faquin[4,5] | Niels J. Rupp[6,7] | Philip Touska[8] | Esther O'Regan[9]

[1]Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

[2]Pathology, Fimlab Laboratories, Tampere, Finland

[3]Guy's Hospital, London, UK

[4]Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts, USA

[5]Harvard Medical School, Boston, Massachusetts, USA

[6]Department of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland

[7]Faculty of Medicine, University of Zurich, Zurich, Switzerland

[8]Department of Radiology, Guy's and St Thomas' Hospital NHS Foundation Trust, London, UK

[9]Department of Histopathology, St. James's Hospital & Dublin Dental Hospital, Trinity College Dublin, Dublin, Ireland

Correspondence
Ivana Kholová, Pathology, Fimlab Laboratories, Arvo Ylpön katu 4, 33520 Tampere, Finland.
Email: ivana.kholova@tuni.fi

Abstract

Cytological specimens play a pivotal role in head and neck nodule/mass work up and diagnoses. The specimens´ importance has grown with the onset of personalized medicine and the routine use of molecular markers in the diagnostic work up. The Updates in Head and Neck Cytopathology Short Course ran during the 35th European Congress of Pathology held in Dublin, Ireland, in 2023 and brought together experts in cytopathology, pathology, and related fields to share their expertise and experience in the field of head and neck cytopathology and its future directions. Topics such as a one-stop clinic, the Milan System for Reporting Salivary Gland Cytopathology, next generation sequencing, and human papilloma virus detection in the head and neck area were covered during the short course. These topics are briefly summarized in the present review.

KEYWORDS
cytology, head and neck, HPV, Milan System, NGS, one-stop clinic

1 | INTRODUCTION

Considering the progress in the field of head and neck pathology and cytopathology, imaging, and molecular pathology in the last decade, it is natural that personalized medicine has brought new challenges to everyday routine work and diagnostic work up of head and neck lesions. With the onset of minimally invasive techniques, the importance of cytopathology has increased. Cytopathology terminologies have been another revolutionary step in cytopathology diagnoses.

The Updates in Head and Neck Cytopathology Short Course ran during the 35th European Congress of Pathology held in Dublin, Ireland, in 2023 and brought together several experts for their insights into various hot topics in the field of head and neck cytopathology and its future directions. Topics included the one-stop clinic, the Milan System for Reporting Salivary Gland Cytopathology in the paediatric population, next generation sequencing, and human papilloma virus detection in the head and neck based on the recent College of American Pathologists consensus.

The present review focused on the topics that were covered during the short course.

2 | HEAD AND NECK FINE NEEDLE ASPIRATION (FNA) CLINICS: IMAGING AND CYTOPATHOLOGY

In the United Kingdom, the last decade has seen increasing adoption of a one-stop diagnostic approach for patients presenting with neck masses to their local Ear, Nose and Throat (ENT) services. The principal rationale for this one stop clinic is to expedite diagnosis and treatment.

The basic process involves patients being seen by an ENT surgeon who, following clinical examination, refers the patient for ultrasound of their neck lump. This scan is carried out by a dedicated head and neck radiologist who will typically evaluate the entire neck using a comprehensive pattern of sweeps. Due to the high spatial resolution of modern ultrasound machines, the location, proximity to critical anatomical structures and nature of the lesion (such as whether it is cystic, solid, vascularised, invasive, or well defined) can be elucidated. It can also be used to identify additional non-palpable lesions that may influence staging. If appropriate, the radiologist will then proceed to carry out a fine needle aspiration (FNA) under ultrasound guidance (which has been shown to significantly improve non-diagnostic rates when compared to non-ultrasound guided sampling). The resultant specimen is prepared by a biomedical scientist for immediate rapid onsite assessment (ROSE) by a cytopathologist[1] (Figure 1).

Using this technique, it may be possible to provide an immediate provisional diagnosis to the referring clinician, based on cytomorphological features alone, for example, typical cases of squamous cell carcinoma (SCC), pleomorphic adenoma and papillary thyroid cancer. Where infection is suspected, material can be sent for microbiological assessment. Where lymphoma is suspected, the cytopathologist can advise the radiologist to carry out a core biopsy, obviating the need to recall the patient for further sampling on another date. In cases where more material is needed to produce a cell block, the radiologist can be asked to undertake additional needle passes. As a result, p16 IHC and HPV in situ hybridisation may be performed in cases of suspected oropharyngeal SCC. The MAML2 gene rearrangement may be identified in cases of mucoepidermoid carcinoma (Figure 2).

However, it is important to be aware of the limitations of the technique. In particular, the cytomorphology of well-differentiated malignancies may be bland and resemble benign lesions, necessitating excision and histological evaluation to confirm malignancy. Examples include basaloid neoplasms of salivary gland and follicular or oncocytic lesions of thyroid. Rare malignancies or variants of common tumours may also prove challenging and auto-immune diseases, such as IgG4 and PD-L1 require core biopsy or excision for diagnosis.[2,3]

Overall, the one-stop approach to the assessment of head and neck masses using a combination of ultrasound-guided FNA (performed by a head and neck radiologist) and ROSE (undertaken by an experienced cytopathologist) represents a gold standard, enabling the skillsets of the specialists involved to be optimally deployed for the benefit of patients.

3 | APPLICATION OF THE MILAN SYSTEM FOR REPORTING SALIVARY GLAND CYTOPATHOLOGY IN PAEDIATRIC POPULATION

After the development of the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC),[4,5] it has been widely

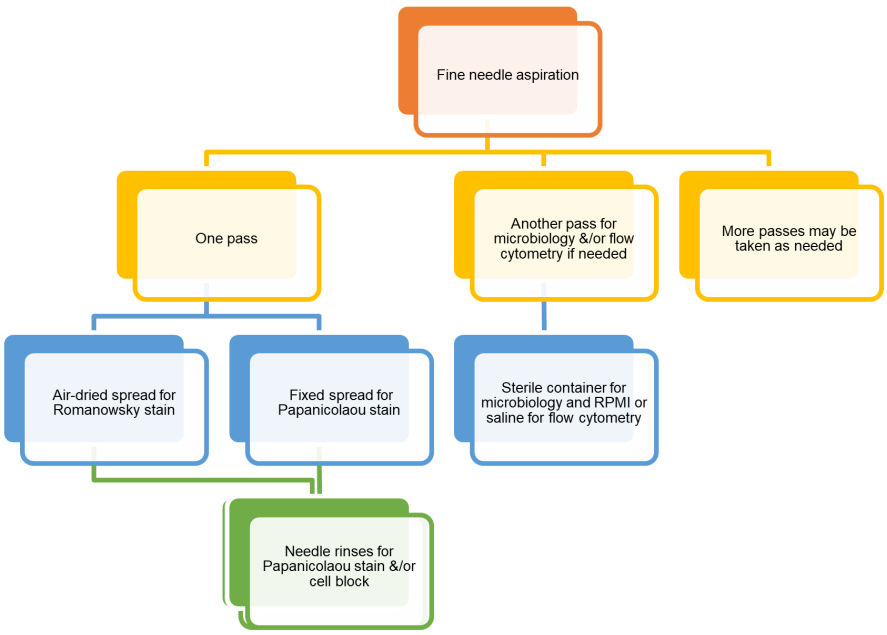

FIGURE 1 Triage of FNA material at Rapid Onsite Evaluation (ROSE) allows needle washings to be sent for appropriate ancillary investigations.

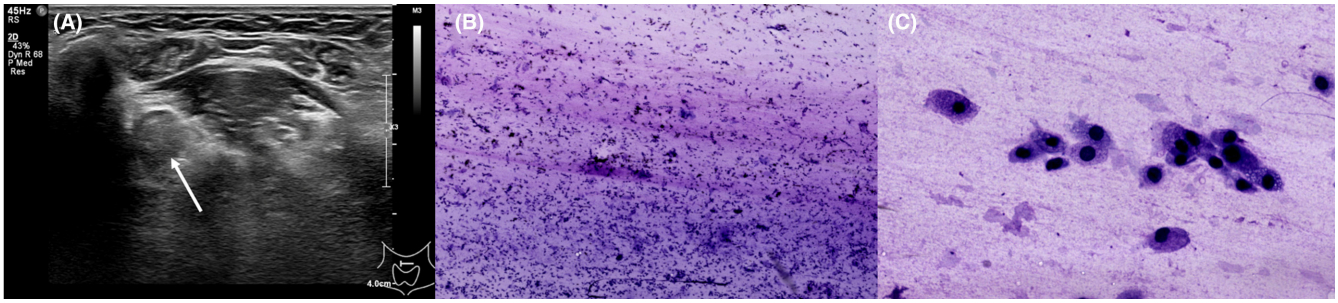

FIGURE 2 (A) Transverse ultrasound image demonstrating an ovoid slightly hypoechoic lesion within the right sublingual gland (arrow) that was subject to FNA. (B) This was suspected to be mucoepidermoid carcinoma due to the presence of mucin on MGG stain at ROSE. (C) Detail of mucinous cells in MGG stain. The slide was used for performing MAML2 gene rearrangement to confirm the diagnosis.

TABLE 1 Summary of international multi-institutional study in paediatric population: Milan System for Reporting Salivary Gland Cytopathology categorisation.

MSRSGC category	Number of cases (%)
I (Nondiagnostic)	49 (10.3)
II (Nonneoplastic)	165 (34.6)
III (AUS)	25 (5.2)
IVa (Benign neoplasm)	131 (27.5)
IVb (SUMP)	36 (7.5)
V (Suspicious)	12 (2.5)
VI (Malignant)	59 (12.4)

Abbreviations: AUS, atypia of undetermined significance; SUMP, salivary gland neoplasm of uncertain malignant potential.

accepted and endorsed by both the cytopathologists and clinicians.[6] However, what is the MSRSGC performance in paediatric population? Are there differences between paediatric and adult populations in the MSRSGC categories and related risks of malignancy in paediatric population? To date, one international multi-institutional study[7] and two institutional studies in paediatric population were conducted.[8,9]

The MSRSGC applicability in the paediatric population was elegantly analysed by an international multi-institutional study[7] that examined 477 fine needle aspiration specimens from paediatric population (age range 0–21 years, 239 males, 238 females). Material originated from 22 institutions from 7 countries. The cases were distributed in the MSRSGC categories as shown in Table 1. Risk of malignancy (ROM) in surgically verified cases is tabulated in Table 2 and compared to adults' data from meta-analyses. The paediatric patients have lower ROM especially in nondiagnostic category.

Additionally, data from two institutional studies have been published.[8,9] Wang et al.[8] analysed retrospectively 104 paediatric cases with 80% sensitivity and 97% specificity. Root cause analysis in nondiagnostic paediatric cases pointed out multifactorial causes as sampling, lesions characteristics, and lack of ultrasound guidance.[10] Satturwar et al. analysed 32 cases with 100% sensitivity and 80% specificity.[8]

Table 2 shows ROMs in the MSRSGC categories in comparison to adults' data from meta-analyses.[11–14] The paediatric patients have lower ROM especially in nondiagnostic category.

4 | NEXT GENERATION SEQUENCING (NGS) FOR GENE FUSION DETECTION IN SALIVARY GLANDS

The molecular characterization of salivary gland neoplasms has made great progress in recent years. The specificity and frequency of these alterations are naturally variable but can nevertheless be diagnostically helpful in a large proportion of cases.

Gene fusions are very closely linked to certain entities and can therefore be used diagnostically, also in cytological preparations. *PLAG1* and *HMGA2* gene fusions are very typical of pleomorphic adenomas (PA; >60–70%),[15] and can thus be used in the appropriate context of a mixed differentiated (epithelial/epithelial-myoepithelial/myoepithelial) neoplasia to support the diagnosis of a cell-rich PA. However, it should be noted that myoepitheliomas,[16] myoepithelial carcinomas,[17,18] and carcinomas ex PA[19] can also show identical *PLAG1* (or *HMGA2*) gene fusions, so that monophasic, myoepithelial neoplasms are more difficult to classify.

MAML2 gene rearrangements are very common (up to 90%, or even more)[15] and highly specific for mucoepidermoid carcinoma (MEC), and its different variants.[20]

In the differential diagnosis of biphasic neoplasms, immunohistochemistry for p63/p40 can be used to corroborate the biphasic differentiation[21]: outer myoepithelial cells positive, inner ductal/epithelial cells negative. The aforementioned PAs, adenoid cystic carcinomas (AdCC), epithelial-myoepithelial carcinomas (EMC), so-called intraductal carcinomas (IDC), and basal cell neoplasms (including basal cell adenoma (BCA) and basal cell adenocarcinoma (BCAC)) encompass the most important biphasic neoplasms. Here, too, the molecular spectrum can be helpful in the diagnosis: PA (*PLAG1* or *HMGA2* gene fusions), AdCC (*MYB::NFIB* or *MYBL1::NFIB* gene fusions; ca. 80%),[22] EMC (*HRAS* point mutations (ca. 80%), occasionally together with *PIK3CA* or *AKT1* mutations[23]; so-called IDC (*NCOA4::RET* and *TRIM27::RET* gene fusions (ca. 50%))[15]; and basal cell neoplasms (activating *CTNNB1* mutations especially in BCA (up to 80%), very rarely in BCAC).[15]

TABLE 2 Comparison of Milan System for Reporting Salivary Gland Cytopathology risk of malignancy in paediatric population to adult population meta-analyses.

	ROM (%)						
	Paediatric population			Adult population			
MSRSGC category	Maleki et al.[7]	Wang et al.[8]	Satturwar et al.[9]	Wang et al.[12]	Jalaly et al.[11]	Gubiotti et al.[13]	Kholova et al.[14]
I (Nondiagnostic)	5.9	0	0	11.4	17.3	17.3	21.0
II (Nonneoplastic)	9.1	11	0	10.9	10.7	14.3	8.1
III (AUS)	35.7	100	0	30.5	32.2	32.7	34.9
IVa (Benign neoplasm)	3.3	6	0	2.8	3.0	2.2	2.4
IVb (SUMP)	31.8	67	0	37.7	37.2	46.8	36.6
V (Suspicious)	100.0	100	100	83.8	84.0	80.0	66.0
VI (Malignant)	100.0	100	0	96.7	98.0	97.1	97.0

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

Overall, the molecular detection of certain alterations by fluorescence in situ hybridisation (FISH) and/or (RNA/DNA) next generation sequencing, together with the morphological/immunohistochemical picture, can contribute important additional information to the better classification of salivary gland neoplasms in cytology.

5 | HPV TESTING OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

Transcriptionally active high-risk HPV (HR-HPV) is a common etiologic agent in the development of oropharyngeal squamous cell carcinomas.[24–28] HPV-associated oropharyngeal squamous cell carcinomas are usually non-keratinizing, frequently metastasize to level II and III cervical lymph nodes, and arise from the specialized crypt lining epithelium of the oropharynx mucosa.[24–28] Patients with these cancers have a significantly improved prognosis and are potential candidates for reduced therapy. This contrasts with patients presenting with traditional alcohol and tobacco-related squamous cell carcinomas (i.e., HPV-independent squamous cell carcinoma) that often harbour mutations in p53, are frequently keratinizing, and typically arise from the surface squamous epithelium. Surgical pathologists and cytopathologists play an important role in determining which head and neck squamous cell carcinomas are positive for HR-HPV.

The College of American Pathologists (CAP) established a committee to establish an evidence-based guideline for HPV testing in head and neck squamous cell carcinomas. The guideline, published in 2017, offers information about which head and neck cancers to test, how to test, and how to interpret the test results.[29] The importance of accurate HPV testing cannot be overemphasized since it will influence a patient's eligibility for clinical trials investigating novel treatment options (e.g., radiotherapy de-escalation or vaccine-based therapies), and it is now integrated into the 8th Edition of the American Joint Committee on Cancer (AJCC) staging manual.[30–32] Currently, the CAP HPV testing guideline committee is working on an updated guideline which is expected to be published in early 2024. The revised guideline will almost certainly advocate for the increased use of HPV-specific testing over p16 testing alone for selected testing scenarios. Regarding FNA specimens where the use of p16 alone on cell block material has been fraught with problems, HPV-specific testing (e.g., RNA ISH and PCR-based liquid tests) may be the solution for providing more accurate test results which will ultimately be more beneficial for guiding patient management.[33–35]

6 | CONCLUSIONS

With rapid emergence of new techniques, approaches, and terminologies, it is important that international cytopathology community at large is educated and skilled to perform fast and comprehensive diagnoses on head and neck lesions with personalized approach.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to conception and design and been involved in drafting the manuscript and/or revising it critically.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Ivana Kholová https://orcid.org/0000-0003-4089-0450
Ashish Chandra https://orcid.org/0000-0003-0339-0086

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