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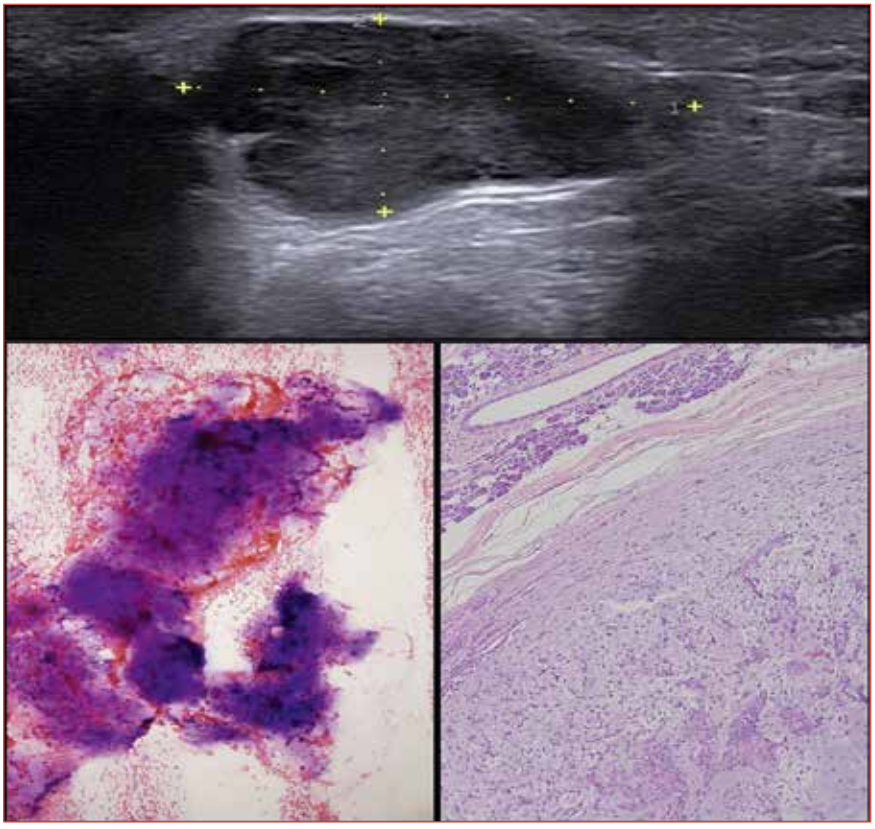
Role of fine needle aspiration biopsy in the diagnosis of pleomorphic adenoma



Cover figure. In the inset at the top, a scan of a parotid pleomorphic adenoma, with the typical posterior acoustic enhancement, which is the most prominent ultrasound feature. In the bottom-left inset the typical cytological appearance of a sample obtained by fine-needle from a pleomorphic adenoma characterized by a proliferation of epithelial and myoepithelial elements embedded in an abundant myxoid matrix. In the bottom-right inset the typical histological appearance with a proliferation of tubular and papillary structures of epithelial elements lined by a layer of myoepithelial cells, also embedded in an abundant myxochondroid matrix.

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Summary

Objective. The aim of the present study is to assess the reliability of fine needle aspiration biopsy (FNAB), an acknowledged diagnostic tool for head and neck lumps, in the diagnosis of pleomorphic adenoma (PA), a common benign salivary tumour.

Methods. We retrieved the FNABs performed at the Otolaryngology Division of a referral centre from September 2017 to September 2023. FNABs were ultrasound (US) guided and followed by rapid on-site evaluation (ROSE). Before surgery, cytological findings were integrated with clinical information to obtain a multiparametric diagnosis (MD).

Results. Of 633 FNAB reports, 413 involved major salivary glands. Among these, 214 patients underwent surgery, and final histopathology confirmed PA in 76 cases. FNAB and multiparametric evaluation yielded specificities of 97% each, and sensitivities of 89.5% and 93.4%, respectively.
Conclusions. US-guided FNAB, performed by head and neck surgeons, associated with ROSE, performed by anatomical pathologist, and MD, is highly reliable for diagnosing PA, thus improving clinical management and prioritisation of cases for surgery. This is particularly relevant considering that PA, even if benign, has a significant rate of malignant transformation and multifocal recurrences, creating notable concerns under both an oncological and functional (facial nerve sparing) points of view.

Key words: fine needle aspiration biopsy, pleomorphic adenoma, multiparametric analysis, salivary glands, cytology

Introduction

Pleomorphic adenoma (PA) is a benign salivary gland tumour (SGT) of epithelial origin and is regarded by many as the most common benign salivary neoplasm, followed by Warthin's tumour (WT) [1,2]. PA may occur at any age but most frequently affects individuals in their fourth to sixth decades of life, with a reported female predominance of approximately 60% [1]. PA mostly arises in the parotid gland representing 55% of all masses and up to 80% of benign tumours [2-6]. More rarely PA may arise in the submandibular and sublingual glands as well as in minor salivary glands of the soft and hard palate, buccal mucosa and nasal cavity [7]. PA typically presents as an encapsulated and well-demarcated mass.

Morphologically, the tumour contains both epithelial and mesenchymal elements with an inner epithelial layer and an outer myoepithelial layer. The connective tissue composition in the tumour varies from mucoid, fibromatous, vascular, and chondroid to myxochondroid, which is associated with a higher risk of recurrence [8]. With incomplete removal or after an intraoperative tumour spillage, the local recurrence rate is up to 45% [6,9]; however, if a histologically confirmed total resection is performed, the recurrence rate is between 1% and 4%.

It has been noted that between 1% and 3 % of primary cases [10] and between 10% and 40% of recurrent cases [7,11] are associated with transformation in carcinoma ex pleomorphic adenoma, with a clear propensity to nerve invasion and most of all distant metastasis [4,5].

In such a clinical framework, of a benign yet insidious disease, the possibility to have a reliable diagnosis to define therapeutic recommendations and relative priority is of paramount importance. For these aims, fine needle aspiration biopsy (FNAB) has been reported to be useful [3,4,12].

The aim of the present study is to further assess the utility and reliability of FNAB in the management of PA, by evaluating sensitivity, specificity and ability to properly exclude malignancy in a well-structured multidisciplinary setting involving both head and neck surgeons and histopathologists, where the main tools available to increasing the power of the assay, and namely ultrasound (US) guidance [2,13-16] and rapid on site evaluation (ROSE) are in place [3,17].

Materials and methods

We conducted an analysis of a monoinstitutional database encompassing all patients who underwent FNAB for head and neck lesions from September 2017 to September 2023, at the multidisciplinary Service created within the Otolaryngology Division of the University Hospital of Sassari, Italy.

Such a multidisciplinary service was conceived basing on the "Lump Clinics" of the Anglo-Saxon health systems (mainly Britain and US) and follows strictly standardised procedures as described below, in order to obtain optimal and reproducible results [2,3,16].

Before each FNAB procedure, pertinent clinical records, such as CT, MRI, US, and PET/CT as well as a detailed clinical history were collected. Specific questions were always formulated regarding onset of symptoms, growth patterns, initial signs and symptoms, personal history of neoplasms or systemic infectious/inflammatory diseases, family history of neoplastic diseases, other clinical conditions, history of tobacco or alcohol use, and concurrent medications. Clinical history collection was followed by physical examination and by a clinically oriented neck US performed by the head and neck surgeon preliminarily to the FNAB.

Up until January 2021, neck US was performed using a Nemio SSA-550A echograph (Toshiba Medical System Corporation®, Otawara, Japan), while afterward, it was conducted using an Acuson NX3 Elite (Siemens Healthcare® s.r.l., Erlangen, Germany) coupled with a 12 MHz 30 mm linear probe. The physical examination consistently involves inspecting and palpating the neck's surface, including the skin, upper airway endoscopy and mucosal surface palpation.

US parameters routinely recorded on standard forms include location, anatomical region, site, and US characteristics (Tab. I) [2].

All FNABs were performed jointly by otolaryngologists and histopathologists following standardised protocols. In brief, the standard procedure involved local disinfection and positioning the US probe on the lesion with the probe's major axis aligned along the needle's intended path, ensuring the needle remained visible within the lesion at all times. Aspiration biopsies were performed using a 27-gauge needle attached to a 20 mL syringe mounted on a Cameco pistol.

The characteristics of the aspirated material, including aspect

Table I. Descriptive statistics, clinical and surgical features of the lesions included in the study.

Features			
Age, (yrs)		Median	52.9
		Range	13-92
Gender, n (%)		Male	42 (38.5)
		Female	67 (61.5)
Disease, n (%)		Primary	107 (98.2)
		Recurrent	2 (1.8)
Subsite, n (%)		Level II	2 (1.8)
		Parapharyngeal	2 (1.8)
		Parotid gland	94 (86.2)
		Submandibular gland	11 (10.2)
Side, n (%)		Left	53 (49)
		Right	56 (51)
Route, n (%)		Transcervical US guided	107 (98.2)
		Transmucosal	2 (1.8)
Ultrasound characteristics, N (%)	**Diameter (mm)**	Median	23.1
		Range	6.7-60
	Echogenicity	Anechoic	1 (0.9)
		Hypo/anechonic	19 (17.7)
		Hypo/isoechoic	29 (27.1)
		Hypoechoic	55 (51.4)
		Iso/hyperechoic	1 (0.9)
		Not reported	2 (1.9)
	Shape	Oval	50 (46.7)
		Polycyclic/lobulated	24 (22.4)
		Round	15 (14.2)
		Spindle-shaped	1 (0.9)
		Not reported	17 (15.9)
	Contour	Irregular	12 (11.2)
		Smooth	45 (42.1)
		Not reported	50 (46.7)
	Margins	Distinct/sharp	80 (74.9)
		Ill-defined	5 (4.7)
		Not reported	22 (20.4)
	Texture	Homogeneous	58 (54.2)
		Inhomogeneous	21 (19.6)
		Not reported	28 (26.2)
	Cystic component	No	49 (47)
		Yes	1 (1)
		Not reported	55 (52)
	Distal phenomena	Distal acoustic enhancement	89 (84.8)
		None	16 (15.2)
	Sonographic palpation: compressible	No	23 (22.1)
		Not done	63 (60.7)
		Yes	18 (17.3)

Table I. *continues.*

Features			
Milan System (for salivary glands only)	I		4 (3.7)
	IVa		102 (93.6)
	IVb		2 (1.8)
	VI		1 (0.9)
FNAB cytology, N (%)	Malignant neoplasms		1 (0.9)
	Not diagnostic		5 (4.6)
	Pleomorphic adenoma		101 (92.7)
	Suspicious for malignancy		2 (1.8)
Final multiparametric diagnosis (clinical + ultrasound features), n (%)	Malignant neoplasms		1 (0.9)
	Not diagnostic		1 (0.9)
	Pleomorphic adenoma		104 (95.5)
	Suspicious for malignancy		2 (1.8)
	Warthin's tumor		1 (0.9)
Surgical treatment, n (%)	Primary		78 (97.5)
	Salvage		2 (2.5)
Time from surgery, months	Median		30.2
	Range		28.9-72.9
Primary site, n (%)	Parotid gland		71 (88.7)
	Submandibular gland		9 (11.3)
Surgery on T, n (%)	Submandibular sialoadenectomy		9 (11.3)
	Superficial parotidectomy		51 (63.7)
	Total/subtotal parotidectomy with VII cranial nerve preservation		20 (25)
Histology, n (%)	Benign		79 (98.8)
	Malignant		1 (1.2)
Histotype, n (%)	**Benign**	Other	1 (1.3)
		Pleomorphic adenoma	76 (96.2)
		Warthin's tumor	2 (2.5)
	Malignant	Adenoid cystic carcinoma	1(100)
Complications, n (%)	**VII cranial nerve - permanent lesion**		1 (1.3)
	VII cranial nerve - postoperative weakness	Grade 2 House- Brackmann scale	7 (46.7)
		Grade 3 House- Brackmann scale	5 (33.4)
		Grade 4 House- Brackmann scale	2 (13.3)
		Grade 5 House- Brackmann scale	1 (6.6)
	Frey's syndrome		7 (10)

(serous, purulent, haematic) and quantity, were consistently recorded. Specimens were immediately stained and submitted to ROSE by a cytopathologist as previously described [2]. If the material was deemed inadequate or insufficient, up to three aspirations were performed. The collected specimens were then processed and analysed following standard guidelines [2].

Cytologic reports of salivary gland lesions routinely incorporate classification according to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) (as shown in Table II) [18]. In case of Class I, before any clinical decisions, a comprehensive reevaluation of cytological findings, clinical history, physical examination, US features, and additional imaging data was performed by otolaryngologists and cytopathologists together, and, in case of consensus, a multiparametric diagnosis, basing upon the multidisciplinary integration and discussion of the above information, was formulated.

We will refer to the setting described above as "Sassari Lump Clinic".

To evaluate the reliability of FNAB and of multiparametric diagnosis concerning PA in our setting, we extracted from the series those cases with a diagnosis of PA in either preoperative evaluation (FNAB and multiparametric) and/or final histopathology report, assessing sensitivity and specificity in the population who underwent surgery.

Follow-up of PA was performed by a morphological baseline imaging (CT or MRI of the head and neck) 6 months after surgery, followed, if negative, by US, performed by an experienced head and neck surgeon or a radiologist, every year for up to 10 years.

Results

A total of 633 FNAB reports were retrieved from our institutional database. A total of 413 of 633 (65.2%) FNABs were performed on major salivary gland lesions. In 214 of 413 (51.8%) major salivary gland cases surgery was performed and a definitive histological report was available. Among the 214 histopathology reports 76 (35.5%) were PA. Of the 413 cytology reports, 101 (24.5%) diagnosed PA, and 3 more cases (104 of 413, 25.2%) had a diagnosis of PA after multiparametric evaluation. Eight cases had a final histopathology report of PA but were referred to surgery with another cytological diagnosis (false negative, FN, for PA): of these, 2 cases were suspicious for malignancy, 5 were non-diagnostic, 1 was diagnosed as a malignancy). Four of the 5 non-diagnostic FNABs were reassigned at multiparametric evaluation, 3 correctly as PA and 1 as WT, so that there were 5 FN cases at multiparametric evaluation. Thus, 109 cases in our series had a cytological/multiparametric or histopathological diagnosis of PA (Fig. 1).

Descriptive statistics of this population (n = 109) with a diagnosis of PA is provided in Table I.

The most constant (~85%) and typical US feature was the distal acoustic enhancement.

Eighty of these cases underwent surgery, and histopathological data were available. In all, 29 cases of 104 (27.9%) with a diagnosis of PA at multiparametric evaluation, for any reason had not undergone

surgery in our institution, yet presumably most are in the waiting list for surgery, as surgery is consistently recommended upon diagnosis of PA. The final histopathology was different than the preoperative report of PA at cytology in 4 of the 80 operated cases (false positive, FP): one resulted to be an adenoid cystic carcinoma (of the submandibular gland), 2 WTs, and one myoepithelioma.

Table II. Diagnostic categories and risk of malignancy (ROM) in the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

Classes	Description	ROM (%)
Class I	Non-diagnostic	25%
Class II	Non-neoplastic	10%
Class III	Atypia of undetermined significance (AUS)	20%
Class IVa	Neoplasm-benign	< 5%
Class IVb	Neoplasm-salivary gland neoplasm of uncertain malignant potential (SUMP)	35%
Class V	Suspicious of malignancy	60%
Class VI	Malignant	90%

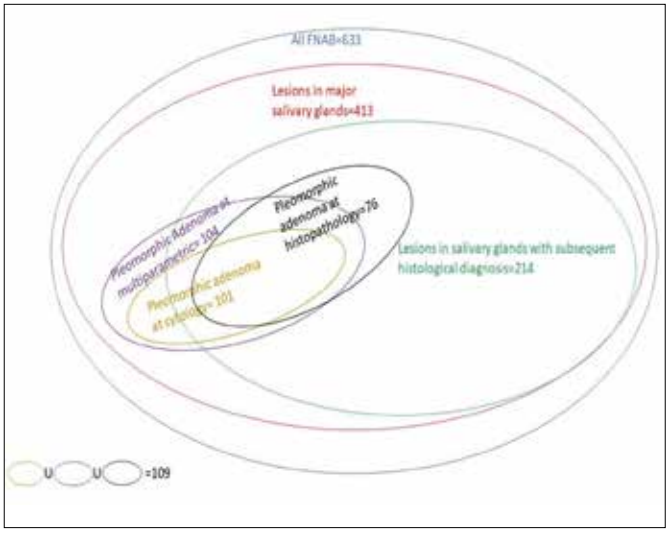

Figure 1. Graphical representation of the diagnostic subgroups within the total number of cases analysed.

In order to obtain a figure of the specificity of cytology in diagnosing PA we decided, in analogy with what we have done in a previous report [2], to take into account for our calculation of the true negative cases only those lesions which occurred in the salivary glands as determined at the preliminary US evaluation, and successively submitted to surgery (n = 214), excluding patients who underwent surgery after FNAB for lesions arising in other head and neck sites. This allows a more conservative estimation of the reliability of the assay.

The specificity (true negatives / [true negatives + false positives]) was 97% for both pure cytology and multiparametric evaluation.

The sensitivity (true positives/[true positives + false negatives]) for detecting PA was 89.5% for pure cytology and 93.4% for multiparametric evaluation.

Most cases (78/80) were primary lesions, and 2 (3%) were typically multifocal recurrent PAs operated elsewhere, re-

spectively 8 and 10 years prior. Notably, the only permanent facial paralysis in the present series, persistent 2 years after the operation despite surgical anatomical preservation of the nerve, was recorded in one of these 2 cases. We proposed a facial reanimation through a Labbè procedure [19], but the patient refused any further surgery. In general, we recorded varying degrees (from II to V according to House-Brackmann) [20] (Tab. I) of transient facial weakness in 15/70 (21.4%) of the remaining parotidectomies, and all experienced complete recovery within 6 months. Seven cases of Frey syndrome were recorded (7/70, 10%); two cases required treatment with botulinum toxin, with remission of pertinent signs and symptoms [21]. No complications were recorded in the 9 submandibular sialoadenectomies, which were all carried out using the recently described submandibular degloving technique [22]. No recurrences were recorded (mean follow-up, 41.3 months).

Discussion

In the described setting, US and US guided sampling were always performed by the same head and neck surgeon who collected the clinical history and performed endoscopic and physical examination, with the advantage, in comparison to radiologists, of the knowledge of surgical anatomy and of specific clinical issues. This practice raises no substantial medico-legal concerns, as in most Western health systems (and in the Italian as well), US is not an exclusive prerogative of radiologists and in many of the other surgical specialties (General Surgery, Gynaecology, Urology, Endocrine surgery, etc.) US is routinely performed by the surgeon.

The present study confirms the usefulness and reliability of US-guided FNAB in managing PA. We are confident that the results are reproducible when the procedural setting previously described [2,3] is adopted.

In this setting, the head and neck surgeon and cytopathologist work side by side: the surgeon, after clinical history, physical examination (including cutaneous inspection), flexible endoscopy, and neck US, selects the most informative sampling site, while the cytopathologist immediately evaluates the specimen by ROSE. All aspirations are performed under direct US guidance; when ROSE indicates insufficient material, up to two additional passes are allowed. If cytology remains inconclusive, the surgeon and cytopathologist merge clinical, US, and cytological data to reach a multiparametric conclusion, provided that the multidimensional information is coherent.

Unlike WT, PA is usually diagnosed on "pure" cytology alone in our Lump Clinic, so the incremental gain in sensitivity from multiparametric analysis is modest for PA but substantial for WT. PA also represents the most frequently resected salivary gland neoplasm in our institution, accounting for 35.5 % of sialoadenectomies, whereas WT remains the most commonly diagnosed lesion overall [2]. This discrepancy is expected: a cytological diagnosis of WT can often be managed conservatively [2], whereas any cytological report of PA mandates surgery for a number of reasons, which also emerge clearly from the present evidence.

First, PA can mimic a malignant neoplasm and vice versa, with a relevant rate of misdiagnosis, also in the present series both at "pure" cytology and at multiparametric diagnosis (see Results and Table I). In our experience, this never happened when the FNAB/multiparametric report was WT [2].

Moreover, unlike WT, PA carries a significant and size-dependent risk of malignant transformation into carcinoma ex pleomorphic adenoma, an aggressive tumour with high metastatic potential [4,23,24].

PAs also exhibit almost invariable growth, and sudden accelerations often herald malignant change [10,11,25].

Surgical excision of PA is notoriously challenging. Spillage of cellular aggregates through capsular rupture is common and represents the principal driver of recurrences [9,24,26]. The tumour's firmness and its intimate relationship with facial-nerve branches, which carve grooves into the expanding mass, further complicate dissection and increase the risks of recurrence and nerve injury, risks that increase with tumour size [2,4,24]. While WT recurrences are rare, usually stemming from gross residual disease, and retain the indolence of the primary tumour, recurrent PA – typically multifocal after spillage – shows higher propensities for further relapse and malignant transformation, often in a scarred field that compromises facial nerve preservation [9,10,23,24,26-28]. Unsurprisingly, the only permanent facial nerve deficit in our cohort followed revision surgery for multifocal PA recurrence.

Early detection of PA therefore remains crucial, as it enables safer, less complex surgery. FNAB is the most effective tool for this purpose, and also helps clinicians prioritise surgical cases among patients presenting with parotid masses.

Conclusions

FNAB, in the context of a properly structured Lump Clinic, is a fundamental tool in the management of salivary neoplasms, and of PA in particular. In these cases, multiparametric evaluation retains added value, though to a lesser extent than in WT [3], in improving diagnostic sensitivity. A certain rate of misdiagnosis with malignancy remains, and is one of the many factors contributing to the mandatory surgical indication in case of a cytological/multiparametric preoperative diagnosis of PA.

An additional value of the Lump Clinic can be seen in training facilities and university hospitals, where, considering the specific surgical challenges of PA, a reliable preoperative diagnosis will allow to acquire fundamental information to rationally assign the procedures to trainees according to their current surgical levels (WT first and PA last), thereby reducing risks for both patients and trainees.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

PT, DR, FB: designed the work; PT, DR, DAM, AM, MAB, AD, CC, SV, LM, RG: acquired and analysed data; AC, JG, FB: drafted, revised and approved the manuscript; PT, LM, JG, FB: final approval of the version to be published. All authors: agree to be accountable for all aspects of the work.

Ethical consideration

This study adhered to the ethical principles outlined in the Declaration of Helsinki. Ethical approval was not mandated by Italian law (GU No. 76, 31 March 2008) due to its observational retrospective design.

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