

ORIGINAL ARTICLE

Use of fluorescent in-situ hybridisation in salivary gland cytology: A powerful diagnostic tool

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Objective: Salivary gland cytology is challenging because it includes a diversity of lesions and a wide spectra of tumours. Recently, it has been reported that many types of salivary gland tumours have specific molecular diagnostic signatures that could be identified by fluorescent in-situ hybridisation (FISH). The aim of the present study was to demonstrate the feasibility and efficiency of FISH on routine cytological salivary gland smears.

Methods: FISH was conducted on 37 cytological salivary gland smears from 34 patients. According to the cytological diagnosis suspected, *MECT1/MAML2* gene fusion and rearrangements of *PLAG1*, *MYB*, or *ETV6* were analysed. The presence and percentages of cells that had gene rearrangements were evaluated. Results were compared with the histological surgical samples, available from 26 patients.

Results: The *PLAG1* rearrangement was observed in 12/20 (60%) cases of pleomorphic adenoma. *MECT1/MAML2* gene fusion was observed in 1:2 mucoepidermoid carcinomas but was not observed in five other tumours (two pleomorphic adenomas, one Warthin's tumour, one mammary analogue secretory carcinoma [MASC] and one cystic tumour). *MYB* rearrangement was observed in 4/4 adenoid cystic carcinomas. *ETV6*-gene splitting identified one MASC.

Conclusion: Overall, FISH had a specificity of 100% and a sensitivity of 66.7%. When FISH and cytological analyses were combined, the overall sensitivity was increased to 93.3%. It can thus be concluded that when the FISH analysis is positive, the extent of surgery could be determined with confidence pre-operatively without needing a diagnosis from a frozen section.

KEYWORDS

ETV6, FISH, *MECT1/MAML2*, *MYB*, *PLAG1*, Salivary gland tumours

1 | INTRODUCTION

Salivary gland tumours are a large group of tumours that are highly diverse and heterogeneous. The 2015 WHO classification described 12 types of benign tumours and 24 types of malignant tumours.¹ Before surgical resection, salivary gland fine needle aspiration (FNA), a method that is easy to perform and is well-tolerated by patients, enables good evaluation of most tumours. However, it represents one of the most challenging cytological fields because of the high

diversity of tumours and their abilities to display cystic change. Indeed, the sensitivity of salivary gland FNA ranges from 66% to 90% and specificity from 86% to 98%.²⁻⁷

In two trained groups of cytologists, cytological and histological diagnoses of malignant tumours were concordant in 80% and 88.8% of cases and cytological and histological diagnoses of benign tumours were concordant in 95.6% and 91.8% of cases.^{4,7} However, these data are variable according to different publications^{5,6} and are dependent upon the learning curve of the observer.

Among the main pitfalls of FNA, mucoepidermoid carcinoma may be missed or misinterpreted as pleomorphic adenoma or squamous cell carcinoma.^{8,9} Adenoid cystic carcinoma may share features with those observed in pleomorphic adenoma and mammary analogue secretory carcinoma (MASC) or may be misinterpreted as acinic cell carcinoma or mucoepidermoid carcinoma.^{10,11}

Recent advances in molecular pathology show that fluorescent in-situ hybridisation (FISH) can identify some molecular diagnostic signatures.¹² For example, approximately 50%-60% of pleomorphic adenomas display a *PLAG1*-gene rearrangement.¹²⁻¹⁵ As pleomorphic adenomas are the most frequent tumours in the parotid gland (59%), detection of a *PLAG1* rearrangement using FNA cytology would aid diagnoses of these benign tumours. The *MECT1/MAML2* gene fusion is caused by a t(11,19) translocation, and can be identified in 35%-65% of mucoepidermoid carcinomas. It is associated with lower histological grades and improved survival.¹⁶⁻¹⁸ Mucoepidermoid carcinomas are the most common primary salivary gland malignancy in both adults and children, and evidence of fusion of these genes can confirm a diagnosis.¹ According to literature, the *MYB-NFIB* fusion transcript is present in 30%-90% of adenoid cystic carcinomas,¹⁹⁻²¹ which are the most frequent form of submandibular gland malignancy.¹ The *ETV6-NTRK3* translocation defines MASC as this translocation is found in 90%-100% of cases.^{22,23} MASC is a new entity that frequently mimics acinar-cell carcinoma or pleomorphic adenoma.²⁴ Although rare, this tumour is mostly observed in children and young adults, and thus could be assessed for *ETV6* rearrangements.

Herein, our experiences are reported with FISH performed on smears from a series of salivary gland tumours and the use of probes to search for *PLAG1*, *MYB1* and *ETV6* rearrangements, and *MECT1/MAML2* gene fusion.

2 | MATERIALS AND METHODS

2.1 | Cytology

Cytological samples from 34 patients were analysed between June 2011 and August 2016. The 34 cases selected for this study had provided an adequate number of slides to enable cytological diagnoses and FISH analyses. The locations of the tumours and clinical data are shown in Table 1. Aspiration of the salivary gland tumour was performed using a 22-gauge needle: at least three smears were realised. One slide was systematically stained with May-Grünwald-Giemsa (MGG). According to the results of the first evaluation, other stains were performed (Papanicolaou and/or Alcian blue).

2.2 | FISH

2.2.1 | Pre-analytic features

To choose the best area on the slide, direct examination of the unstained slide was performed on samples taken from patients no. 1-23 at the beginning of the study. Later, because of difficulties encountered in avoiding stromal areas (which caused strong

background staining), a RAL 555 kit was used (RAL diagnostics, Martillac, France) to lightly stain the cells (ie samples from patients no. 24-32).

Briefly, the slides were methanol-fixed for 5 s, stained only with methylene blue for 5 s, and then immediately rinsed in tap water.²⁵ Direct examination under a microscope enabled us to choose an area with individual, non-overlapping cells devoid of a matrix. In two cases (ie patients no. 33 and no. 34), FISH was performed on Papanicolaou- and MGG-stained slides, respectively, because not enough material was available.^{26,27} In these two cases, the stained slides were unmounted and left in acetone for a few hours.

2.2.2 | FISH probes

Four different probes were used in this study. The probes used to detect a *PLAG1* rearrangement, a *MYB* rearrangement or the *MECT1/MAML2* gene fusion were synthesised in the haematological laboratory (Pr Sié) at our institution (IUCT-Oncopole) (Table 2). Bacterial artificial chromosomes were chosen from the GRCh38.p2 database (<http://www.ensembl.org/index.html>) and were tagged by nick translation with green (FITC) or red (Texas Red) fluorochrome (SpectrumGreen and SpectrumRed; Abbott, Downers Grove, IL, USA) (Table 2). A normal hybridisation pattern was observed when the different sets of probes were tested on negative control tissue slides.

The probes were also tested on positive controls (tissues from salivary glands tumours with gene rearrangements), as follows.

- A *MYB* positive control, with t(X;6)(p11;q23) /*GATA1-MYB* gene fusion.
- *PLAG1* rearrangement: histological section of salivary gland pleomorphic adenoma.
- *MECT1 /MAML2* gene fusion: histological section of salivary gland mucoepidermoid carcinoma.

PLAG1 and *MYB* probes were break-apart probes; the *MECT1/MAML2* probes were dual-fusion probes.

The *ETV6* probes from Dako, Glostrup, Denmark (break-apart probes) were used to detect rearrangements of the *ETV6* gene in MASC.

2.2.3 | FISH process

The slides were fixed in a methanol-acetic-acid solution (3:1 v/v) for 30 min. After air-drying, the slides were immersed for 2 min in 2X saline sodium citrate (SSC) solution heated to 72°C. The slides were then put on a hybridiser at 37°C and covered with pepsin solution (Histology FISH Accessory Kit; Dako) for 10 min. After washing in 1X phosphate-buffered saline (PBS), the slides were post-fixed in 3.7% formaldehyde and washed again, twice, with 1X PBS. The slides were then dehydrated in successive baths of ethanol at increasing concentrations (70%, 80%, 90% and 100%).

After air-drying, the probes (*PLAG1* probes, *MYB* probes, *MECT1/MAML2* probes or *ETV6* probes [Dako]) were dripped onto defined

TABLE 1 Clinical, cytological and pathological data from the patients

Number	Sex	Age	Localisation	Cytological diagnosis	FISH probe	Presence of gene rearrangement or translocation	% of rearranged cells (number of rearranged cells / total number of cells)	Surgery	Histological diagnosis
1	M	55	Parotid	PA	PLAG1	Yes	45 (25/55)	Yes	PA locally invasive
2	M	48	Parotid	PA with atypia	PLAG1	Yes	35 (28/80)	Yes	PA with carcinoma foci
3	M	46	Parotid	Cellular-rich PA	PLAG1	No	0 (0/60)	Yes	Cellular PA
4	F	91	Parotid	MEC	MECT1/MAML2	Yes	21 (21/100)	No	
5	F	69	Parotid	AdCC	MYB	Yes	90 (45/50)	Yes	AdCC
6	M	15	Submandibular gland	PA cannot exclude MEC	MECT1/MAML2	No	5 (3/62)	Yes	PA
7	M	74	Parotid	Basal cell adenoma	MYB	No	6 (2/33)	Yes	Epithelial-Myoepithelial carcinoma
8	M	53	Parotid	PA	PLAG1	No	2.5 (2/83)	Yes	PA
9	F	74	Parotid	PA	PLAG1	Yes	20.4 (11/54)	Yes	PA
10	F	43	Parotid	Warthin's tumour but clinical suspicion of MEC	MECT1/MAML2	No	0 (0/32)	Yes	Warthin tumor
11	F	52	Parotid	PA	PLAG1	No	5 (2/39)	Yes	PA
12	M	46	Parotid	PA	PLAG1	Yes	97 (97/100)	Yes	MEC on PA
13	M	62	Parotid	PA	PLAG1	Yes	100 (64/64)	Yes	PA
14	F	24	Parotid	Cellular-rich PA	PLAG1	Yes	60 (11/17)	Yes	Cellular PA
15	F	40	Submandibular gland	PA	PLAG1	Yes	47 (18/38)	No	
16	M	48	Parotid	PA	PLAG1	No	7.5 (14/186)	Yes	PA
17	M	37	Parotid	PA	PLAG1	No	4 (4/100)	Yes	PA
					MYB	No	5.4 (5/92)		
18	M	40	Parotid	Cellular-rich PA	PLAG1	Yes	61 (92/150)	Yes	Cellular PA
19	F	11	Parotid	Myoepithelial tumour	MECT1/MAML2	No	7 (7/100)	Yes	MASC
20	M	53	Parotid	MASC	ETV6	Yes	69 (40/58)	Yes	MASC
21	M	76	Parotid	PA	PLAG1	Yes	36.9 (38/103)	No	
22	F	38	Parotid	AdCC	MYB	Yes	53 (53/100)	Yes	AdCC
					PLAG1	No	4 (4/100)		
23	M	45	Submandibular gland	PA	PLAG1	Yes	35 (17/50)	No	
24	M	60	Parotid	Adenocarcinoma NOS	ETV6	No	77 (5/65)	No	Metastatic lung adenocarcinoma
25	M	55	Parotid	PA	PLAG1	Yes	68 (68/100)	No	
26	M	59	Parotid	Cystic tumour	MECT1/MAML2	No	8 (4/46)	No	

(Continues)

TABLE 1 (Continued)

Number	Sex	Age	Localisation	Cytological diagnosis	FISH probe	Presence of gene rearrangement or translocation	% of rearranged cells (number of rearranged cells / total number of cells)	Surgery	Histological diagnosis
27	F	41	Palate	PA	PLAG1	Yes	66 (10/15)	Yes	PA
28	M	25	Parotid	MEC	PLAG1	No	4 (2/50)	Yes	MEC
29	F	41	Parotid	PA	MECT1/MAML2	No	1 (1/100)	Yes	PA
30	M	79	Mandibular lesion	AdCC	MYB	Yes	80 (80/100)	Yes	AdCC
31	F	29	Parotid	PA	PLAG1	No	7 (7/100)	Yes	PA
32	F	26	Parotid	PA	PLAG1	No	3 (3/100)	No	
33	F	85	Cervical lymph node	AdCC metastasis	MYB	Yes	30 (18/60)	Yes	AdCC
34	F	61	Parotid	Suspicious for MEC	MECT1/MAML2	No	7.9 (14/178)	Yes	PA

PA, pleomorphic adenoma; MEC, mucoepidermoid carcinoma; AdCC, adenoid cystic carcinoma; MASC, mammary analogue secretory carcinoma; ACC, acinic-cell carcinoma.

areas of the slides and the slides were placed on a hybridiser for 5 min at 82°C, followed by 18 h at 37°C. The following day, the slides were immersed in 1X SSC solution heated to 72°C for 5 min, and then in 1X SSC solution at room temperature for 5 min. A drop of DAPI (fluorescent-mounting medium; Dako) was placed on each defined area of each slide. The slides were kept at 4°C for at least 15 min before being visualised.

The first slides prepared in this study were read with a fluorescence microscope (Carl Zeiss, Göttingen, Germany) and cells with or without a rearrangement were counted directly. Since April 2014, the slides have been scanned with a slide scanner (Panoramic 250 FlashII, 3DHisTech; Sysmex, Villepinte, France). Fluorescent signals were visualised and cells were counted using Panoramic viewer software (3DHisTech). As previously described, a cut-off value of 10% of cells with a gene rearrangement was used for the interpretation.¹³

2.3 | Statistical analyses

Sensitivity and specificity were calculated for the FNA cytology using the postoperative histological diagnosis as the gold standard. If the histological assessment resulted in a diagnosis of malignancy, it was classified as a positive result, whereas a benign or non-neoplastic lesion was considered negative.²⁸ Similarly, sensitivity and specificity of FISH analyses were calculated for patients with a histological diagnosis (or with a cytological diagnosis when a histological diagnosis was unknown). The sensitivity and specificity were calculated as follows:

$$\text{Sensitivity} = \text{True positive} / (\text{True positive} + \text{False negative})$$

$$\text{Specificity} = \text{True negative} / (\text{False positive} + \text{True negative})$$

Combined sensitivity and specificity of the cytological diagnosis and FISH analyses were also calculated. We thus considered that cytological diagnosis and FISH analysis are two tests applied “in parallel” so that a positive result from either test resulted in the overall result being classified as positive.²⁹ The combined sensitivity and specificity of the cytological diagnosis and the FISH analyses were calculated as follows:

$$\begin{aligned} \text{Combined sensitivity of cytological diagnosis and FISH analyses} \\ = \text{sensitivity of cytological diagnosis} + \text{sensitivity of FISH analysis} \\ - (\text{sensitivity of cytological diagnosis} \times \text{sensitivity of FISH analysis}); \end{aligned}$$

$$\begin{aligned} \text{Combined specificity of the cytological diagnosis and FISH analyses} \\ = \text{specificity of the cytological diagnosis} \\ \times \text{specificity of the FISH analyses} \end{aligned}$$

3 | RESULTS

Thirty-seven FISH analyses were performed on cytological smears from 34 patients. These cytological samples originated from FNAs of the parotid for 28 patients, the submandibular gland for three patients, a mandibular lesion for one patient, the palate for one patient and a cervical lymph node for one patient. The patients' median age was 48 years (range: 11-91), and the male-to-female

TABLE 2 FISH probes designed and produced in our institution

Gene, chromosome localization, clones	Position compared to the gene	Fluorochrome
MYB, 6q23		
RP11-104D9	5'	Spectrum Red
RP11-905P20	3'	Spectrum Green
PLAG1, 8q12		
RP11-585J20	3'	Spectrum Red
RP11-446E9	3'	Spectrum Red
RP11-140I16	5'	Spectrum Green
RP11-342K10	5'	Spectrum Green
MECT1 / MAML2, 19p13/11q21		
RP11-908B10	Cover MECT1	Spectrum Red
CTD-2643B8		Spectrum Red
CTD-2546B19		Spectrum Red
RP11-338H14	Cover MAML2	Spectrum Green
RP11-7D4		Spectrum Green
RP11-205M24		Spectrum Green
RP11-111I14		Spectrum Green
RP11-16K5		Spectrum Green
RP11-8N17		Spectrum Green
RP11-580A13		Spectrum Green

ratio was 19/15. The results of the FNA cytology, the FISH and the histopathology are shown in Table 1. Some examples of cytological and FISH results are presented in Figures 1-4.

Salivary gland FNA enabled cytological diagnosis of pleomorphic adenoma in 21 patients. Of these, a diagnosis of pleomorphic adenoma was confirmed by histology in 15 patients. For one patient (patient no. 12), the histological analysis diagnosed carcinoma ex-pleomorphic adenoma. Five patients (nos 15, 21, 23, 25, 32) did not undergo surgery in our institution and, thus, we do not have a histological diagnosis for them.

A *PLAG1* rearrangement was observed in 12 patients. Interestingly, as has been previously described, a *PLAG1* rearrangement was observed in one case of carcinoma ex-pleomorphic adenoma (patient no. 12), which was diagnosed by the histological analysis.^{13,20,30,31} Cytological re-examination of the slides from this patient confirmed the absence of characteristics of carcinoma on the cytological slides,

which contained only pleomorphic adenoma features. This, thus, resulted in a false-negative result.

FISH analysis was negative for a *PLAG1* rearrangement in 10 cases. Among these, eight patients presented with a typical cytology of pleomorphic adenoma and two patients were diagnosed with other tumours (one mucoepidermoid carcinoma [patient no. 25] and one case of adenoid cystic carcinoma [patient no. 22]) (Table 1). A median value of 53.5% of cells with a split signal was noted in *PLAG1*-positive tumours (defined as a tumour with >10% of cells with a split signal) and a median value of 4% of cells with a split signal was noted in *PLAG1*-negative cases (<10% of cells with a split signal).

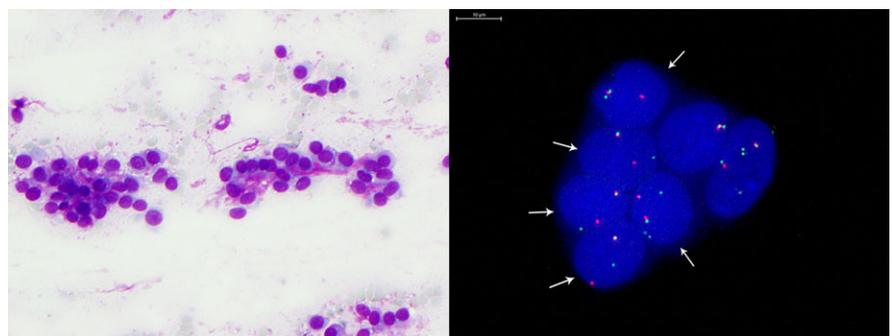
Two mucoepidermoid carcinomas diagnosed by salivary gland FNA were analysed by FISH. One case (no. 4) showed a *MECT1/MAML2* gene fusion in 21% of the cells. As a result of the advanced age of this patient, surgery was not performed, but the cytological features were strongly evocative. One case (no. 28) did not show *MECT1/MAML2* gene fusion, even though this corresponded to a low-grade mucoepidermoid carcinoma after histological analysis. However, a *MECT1/MAML2* gene fusion was not observed in approximately 25% of low- and intermediate-grade tumours.¹⁶

The *MECT1/MAML2* gene fusion was not observed in five other types of tumours (two pleomorphic adenomas, one Warthin's tumour, one MASC and one cystic tumour), as has been similarly described in the literature.³²

A *MYB* rearrangement was found in all four cases of adenoid cystic carcinoma diagnosed from the cytological analysis, including one case of lymph-node metastasis from a submandibular adenoid cystic carcinoma. Depending on the tumour, 30%-90% of cells with a gene rearrangement were found. The *MYB* gene was not rearranged in two other tumour types (one epithelial-myoepithelial carcinoma and one pleomorphic adenoma).

Because MASC tumours are rare, an *ETV6* gene rearrangement was only found to be positive in one salivary gland FNA where a diagnosis of MASC was suspected after the cytological analysis. The histological analysis, which was realised later, confirmed this diagnosis. Another case was diagnosed by histology in our laboratory that had been previously misinterpreted after cytological analysis. The cytological slides from this patient were analysed in our laboratory but came from another laboratory. When the histological diagnosis was made, these cytological slides were no longer available in our

FIGURE 1 Pleomorphic adenoma is characterised by plasmacytoid cells mixed with the fibrillary matrix, stained magenta in the left picture ([May-Grünwald-Giemsa MGG] staining). Fluorescent in-situ hybridisation (FISH) revealed rearrangement of the *PLAG1* gene, as indicated by a split between the green and red signal (arrows) (right picture). The two pictures are from the same patient.



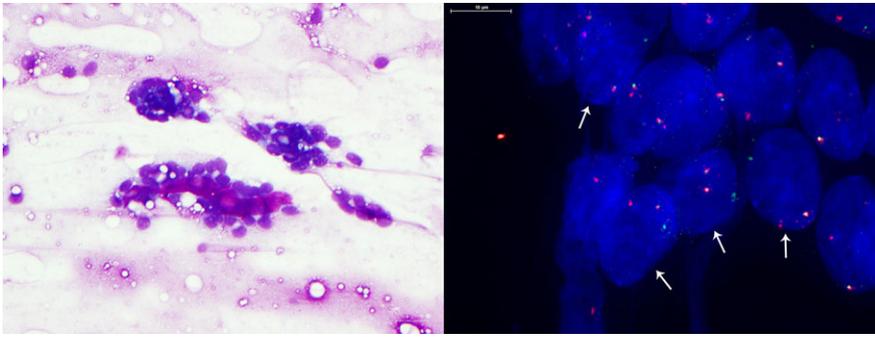


FIGURE 2 Adenoid cystic carcinoma is characterised by basal cells surrounding a finger-like matrix (left picture, May-Grünwald-Giemsa [MGG] staining). Fluorescent in-situ hybridisation (FISH) revealed rearrangement of the *MYB* gene as indicated by a split between the green and red signal (arrows) (right picture). The two pictures are from the same patient

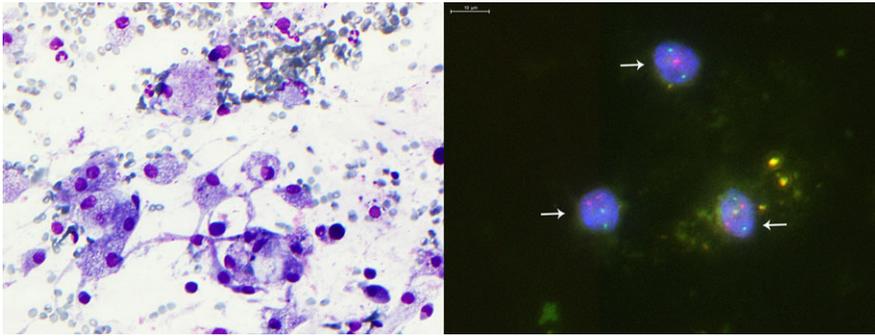


FIGURE 3 Mucoepidermoid carcinoma is characterised by mucus-secreting cells with a vacuolated cytoplasm (left picture, May-Grünwald-Giemsa [MGG] staining). Fluorescent in-situ hybridisation (FISH) revealed no rearrangement between the *MECT1* gene and the *MAML2* gene (right picture). The green and the red signal are separated (arrows). The two pictures are from the same patient

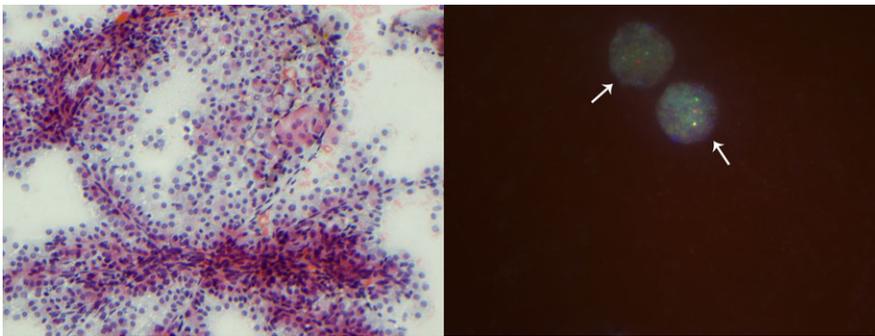


FIGURE 4 Mammary analogue secretory carcinoma (MASC) is characterised by medium-sized cells distributed along fibro-vascular cores (left picture, Papanicolaou staining). Fluorescent in-situ hybridisation (FISH) revealed rearrangement of the *ETV6* gene as indicated by a split between the green and red signal (arrows) (right picture). The two pictures are from the same patient

laboratory. Thus, FISH to detect the *ETV6* gene rearrangement had not been performed on this cytological sample.

Overall, FISH performed on cytological smears had a sensitivity of 66.7% and a specificity of 100%. In the present study, FNA cytology showed a sensitivity of 80% and a specificity of 94.1% which are both concordant with previous studies.^{7,28,33,34} The combined sensitivity and the combined specificity for cytological diagnoses and FISH analyses were 93.3% and 94.1%, respectively.

4 | DISCUSSION

The classic presentations of tumours are often easily recognised after cytological analyses by an experienced cytologist. However, owing to the overlapping cytological aspects between tumours, diagnosis may be challenging, especially in cases with few cells. Immunohistochemistry may represent a helpful tool but necessitates several antibodies applied on several slides, which are usually not available for cytological FNA smears. FISH, when positive, has the advantage

of being fully specific for a tumour and, in some cases such as mucoepidermoid carcinoma, can indicate a better prognosis.

In this study, only cytological FNA smears for FISH analysis were used because clinicians at our institution are used to sending us cytological FNA smears. However, it is possible to use liquid-based cytological (LBC) preparations for FNA cytological diagnoses. Different publications report that these two different cytological preparations do not affect cytological interpretations.^{7,35,36} A few papers have reported that FISH is possible on ThinPrep® slides obtained from different tumours.^{37,38} However, to our knowledge, this has not been shown for salivary gland tumours. In addition, this method would require the entire stained ThinPrep slides to be scanned after cytological diagnosis, and to then unmount the slides and perform the FISH analysis. All these steps can markedly increase the time of analysis.

For salivary gland tumours, Pusztaszeri et al. used a Cytoblok from an LBC preparation and then performed FISH analysis on the Cytoblok.¹² This type of FISH analysis is similar to the FISH analysis performed on histological material described in the literature. The

originality of the present study is to show that FISH analysis is possible on cytological smears, which is easy-to-realise and is a low-cost cytological preparation. Moreover, this technique was appropriate for our daily practice as most of our FNA salivary gland samples are received as air-dried smears.

For the routine use of FISH on cytological FNA smears, we had to modify our standard protocol. First, we were surprised by the strong background level of staining caused by the abundant extracellular matrix that is frequently observed in these types of tumours. Pretreatment of the slides with a mucolytic solution (Dithiothreitol; Sigma-Aldrich, L'Isle d'Abeau Chesnes, France) failed to improve the results. However, the results were improved when an area on the slides was selected where the cells were separated, and there was no matrix. To do this, we used Papanicolaou or MGG-stained slides or slides stained with modified RAL (without eosin staining, which increases the background stain). In daily practice, when such conditions are not met, we performed immunocytochemistry when possible.

A cytological diagnosis of pleomorphic adenoma is often evident when ductal and myoepithelial cells are admixed with an abundant magenta chondromyxoid fibrillary matrix on MGG-stained slides. However, difficulties may arise when hyaline globules are observed, as they may mimic adenoid cystic carcinoma. Pathologists should be aware that a *PLAG1* rearrangement is still present in carcinoma ex-pleomorphic adenoma. This rearrangement is then not synonymous with benignity but marks only the presence of a pleomorphic adenoma, which could be benign or malignant.¹

Some cases of pleomorphic adenoma can be challenging to diagnose when myoepithelial cells are predominant, and chondromyxoid stroma is lacking.³⁹ In these cases, the presence of a *PLAG1*-gene rearrangement indicates a pleomorphic adenoma. In addition, rearrangements of *HMGA2* have also been described in pleomorphic adenoma and carcinoma ex-pleomorphic adenoma.^{13,40} As this rearrangement is found in only 10% of pleomorphic adenomas, we decided to not search for this rearrangement, which is less frequent than the *PLAG1* rearrangement.

Another challenging diagnosis for cytologists is represented by mucoepidermoid carcinoma. Because of the mucin secretion, FNAs are often not very cellular, and sometimes glandular goblet cells are lacking. Differential diagnoses include benign cysts (especially in the submandibular gland) or mucin-rich variants of salivary duct carcinoma.⁹ Indeed, the oncocyctic variant of mucoepidermoid carcinoma may be impossible to differentiate from other oncocyctic tumours.

The *MECT1/MAML2* gene fusion is specific to mucoepidermoid carcinoma and is observed in 35%-65% of cases.³² Translocation is observed in 75% of low- and intermediate-grade tumours and in 46% of high-grade tumours.¹⁶ Because the *MECT1/MAML2* gene fusion is specific to mucoepidermoid carcinoma, we performed *MECT1/MAML2* FISH on five other tumours (patients no. 6, 10, 19, 26, 34) that had atypical cytological presentations to eliminate a possible diagnosis of mucoepidermoid carcinoma. As expected, FISH was negative in all these cases. Some cases of Warthin's tumour

have been reported to have a *MECT1/MAML2* gene fusion, but they probably correspond to the recently published entity "Warthin-like mucoepidermoid carcinoma".⁴¹ Moreover, patients with "Warthin-like mucoepidermoid carcinoma" have a prognosis of mucoepidermoid carcinoma: this type of entity justifies the use of FISH to refine a diagnosis of FNA.

Adenoid cystic carcinomas are often relatively easy to diagnose in their cribriform presentation. The presence of an *MYB-NFIB* fusion oncogene is indicative of a diagnosis.⁴² Persson et al. initially showed that this translocation is a hallmark of adenoid cystic carcinoma and found that it was positive in a series of 6/6 head-and-neck, 4/4 breast and one metastatic localisation.²⁰ Subsequently, Mitani et al. demonstrated *MYB* rearrangement in only 28% of primary and 35% of metastatic adenoid cystic carcinomas in a larger sample (89 cases).¹⁹ Brill et al. showed that the *MIB-NFIB* fusion transcript was present in up to 86% of tumours when frozen material was used.⁴³ In our series, we observed *MYB* rearrangement in 4/4 of the adenoid cystic carcinomas. In this way, when positive, FISH analysis can confirm a diagnosis, even on samples with few cells: a suspected malignancy from an FNA can then be verified or rejected using FISH to assess *MYB* rearrangement.

MASC is a newly recognised tumour that can mimic acinic-cell carcinoma.²² Positive staining with Mammaglobin and pS100 can suggest this diagnosis.⁴⁴ Indeed, rearrangement of the *ETV6* gene is specific to MASC. In our series, one case (#20) was correctly diagnosed by cytology, and the *ETV6* rearrangement was present. Another case (no. 19) was misdiagnosed as a myoepithelial tumour by cytology: FISH was not performed (because the slides used for this diagnosis were unavailable in our laboratory) but was positive in histology.

In conclusion, FISH is a useful tool to characterise some frequently observed salivary gland tumours, directly on cytological smears. However, this only applies to positive results as a negative result cannot be used to exclude a specific diagnosis. In addition, the combined sensitivity of FISH analysis and FNA cytology is greater (93.3%) than the sensitivity of FISH (66.7%) or FNA cytology (80%) alone, suggesting that FISH analysis combined with FNA cytology could improve the diagnosis of salivary gland tumours.

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CONFLICT OF INTEREST DISCLOSURES

The authors have no disclosures to declare.

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