The Impact of FNAC in the Management of Salivary Gland Lesions: Institutional Experiences Leading to a Risk-Based Classification Scheme

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BACKGROUND: Fine-needle aspiration cytology (FNAC) has proven its value as an essential step in the diagnosis of salivary gland lesions. Although the majority of salivary gland lesions, especially those that are common and benign, can be diagnosed with ease on FNAC, limited cellularity and morphologic lesion heterogeneity can pose diagnostic challenges and lead to false-positive and false-negative diagnoses. This study presents the institutional experience of FNAC of salivary gland lesions from 2 academic centers. **METHODS:** A retrospective analysis was conducted on 1729 salivary gland FNAC specimens that were diagnosed over an 8-year period from January 2008 to March 2015. All samples were processed either with liquid-based cytology alone or in combination with air-dried, Diff-Quik–stained or alcohol-fixed, Papanicolaou-stained smears. **RESULTS:** Surgical excision was performed in 709 of 1749 FNACs (41%) that were diagnosed as nondiagnostic/inadequate (n = 29), benign (n = 111), neoplasm (n = 453), atypical (n = 15), suspicious for malignancy (n = 28), and malignant (n = 73). The overall concordance between cytologic and histologic diagnoses was 92.2%, with 91.8% concordance in the benign category and 89.5% concordance in cases diagnosed as suspicious for malignancy and malignant. The most frequent benign and malignant lesions were pleomorphic adenoma and squamous cell carcinoma, respectively. There were 46 false-negative and 13 false-positive results, leading to an overall specificity of 97.6% and diagnostic accuracy of 91.3%. **CONCLUSIONS:** FNAC is a reliable diagnostic modality for the diagnosis and management of salivary gland lesions based on its high specificity and diagnostic accuracy. ***Cancer Cytopathol* 2016;124:388-96.** © *2016 American Cancer Society.*

KEY WORDS: fine-needle aspiration cytology; liquid-based cytology; malignancies; salivary gland lesions.

INTRODUCTION

Fine-needle aspiration (FNA) cytology (FNAC) can be easily used in an outpatient setting to diagnose and manage superficial salivary gland lesions.[1–6] This role for FNAC has gained wide acceptance among clinicians because of easier accessibility of the lesions involving salivary glands (especially those affecting the parotid and submandibular glands) and the difficulties in distinguishing between benign and malignant lesions solely based on radiologic evaluation.

The cytologic features of salivary gland lesions in FNAC specimens have been well defined and are described in detail in the literature. However, many studies have also pointed to the lack of optimum diagnostic sensitivity and specificity of FNAC in achieving a conclusive differentiation between some benign and malignant

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Received: December 28, 2015; **Revised:** February 3, 2016; **Accepted:** February 12, 2016

Published online March 9, 2016 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cncy.21710, wileyonlinelibrary.com

entities.[7–10] Other studies have emphasized the high cost-effective value of this diagnostic procedure, because it leads to a reduction in unnecessary surgery.[11,12] It has been demonstrated that FNAC of salivary gland lesions is associated with high diagnostic accuracy in benign rather than malignant lesions because of the heterogeneous morphology and architecture of malignant tumors, especially in patients who have biphasic neoplasms.[13–15]

In this article, we report our experience from 2 academic institutions with a large cohort of salivary gland FNAC specimens that were processed with both conventional and liquid-based cytology. Our objective was to appraise the importance of a correct cytologic diagnosis, regardless of the cytologic preparation used, and discuss the need for a tier-based classification scheme in light of the risk of malignancy, false-negative results, and false-positive results.

MATERIAL AND METHODS

We performed a retrospective, computerized search of all salivary gland FNAC cases recorded between January 2008 and March 2015 at the Catholic University "Agostino Gemelli" Hospital (Rome, Italy) (Catholic University) and between January 2009 and June 2014 at the Hospital of the University of Pennsylvania (Philadelphia, Pa) (HUP). This resulted in a case cohort of 1729 salivary gland lesions (1123 from Catholic University and 606 from HUP) diagnosed during the study period. Histopathologic follow-up was available for 709 cases (426 from Catholic University and 283 from HUP). At both institutions, the majority of nodules were evaluated and biopsied under ultrasound (US) guidance by clinicians and radiologists. All FNAC specimens from Catholic University were processed using the liquid-based cytologic (LBC) method with ThinPrep 5000 processing (Hologic Company, Marlborough, Mass) and alcohol-fixed Papanicolaou stain; whereas, at HUP, the specimens were processed using the standard smear and respective staining methods (air-dried, Diff-Quick smears; alcohol-fixed, Papanicolaou-stained smears; and LBC).

All FNAs (usually 2 passes for each lesion) were performed with 25-gauge to 27-gauge needles at both institutions. The specimens from Catholic University were collected using passes without rapid on-site evaluation, with 1 pass for conventional slides and the second pass for LBC. Rapid on-site evaluation was performed by cytopathologists for all specimens at HUP.

The lesions ranged in size from 0.4 cm to 6.0 cm in greatest dimension. All subcentimeter lesions were discovered during radiologic screening for causes unrelated to salivary glands. At Catholic University, all patients had been appropriately informed about the LBC procedure for processing their aspiration samples, and a written informed-consent form was signed by all study participants. Our study was independently evaluated and approved by the institutional review boards at both institutions.

For the purposes of risk stratification and descriptive analysis, the cytology diagnoses were grouped into 4 main categories: 1) nondiagnostic, 2) nonneoplastic (including sialadenitis, lipoma, and cysts), 3) benign neoplasms (including Warthin tumor, pleomorphic adenoma [PA], oncocytoma, myoepithelioma, basal cell adenoma, and adenoma with myoepithelial/basal cell features), and 4) malignant neoplasms (including atypical lesions, suspicious for malignancy, and primary and metastatic lesions). Specifically for specimens that either had basaloid features or contained a distinct myoepithelial component, the cytologic interpretation was based on those already described in the literature.[10,16–19]

Statistical Analysis

Descriptive statistical analysis (including sex, age, laterality, nodule size, type of surgery, and histologic diagnosis) was performed using a commercially available statistical software package (SPSS version 23.0; IBM Corporation, Chicago, Ill) for Windows (Microsoft Corporation, Redmond, Wash). The histologic diagnosis was considered the gold standard for statistical analysis. Comparison of categorical variables was performed with the Z-Test Calculator (SPSS Microsoft package) for 2 population proportions using a 2-tailed hypothesis test. P values $< .05$ were considered statistically significant. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated based on cytohistologic correlation in the diagnosis of salivary gland lesions.

RESULTS

The study cohort included 1729 specimens (1123 from Catholic University and 606 from HUP) that were obtained between January 2008 and March 2015, including 709 specimens (41%) that had corresponding surgical

TABLE 1. Clinicopathologic Features of 1729 Salivary Gland Fine-Needle Aspiration Cases

	R/P Cytology Diagnostic Categories			
			Neoplastic	
Features	Inadequate	Nonneoplastic[a]	Benign[b]	Malignant[c]
No. of cases (total no.)	38/63 (101)	175/148 (323)	787/287 (1074)	123/108 (231)
No. of males/females[d]	43/58	168/155	593/481	136/95
Sites, no. of cases				
Parotid[d]	93	271	1027	219
Submandibular[d]	5	31	28	7
Minor salivary gland[d]	3	21	19	5
Mean size, cm[d]	2.25	2.73	2.35	2.28
Histologic follow-up: 709 cases, no. (total no.)[d]	15/14 (29)	63/48 (111)	294/159 (453)	54/62 (116)

Abbreviation: R/P, Catholic University "Agostino Gemelli" Hospital/Hospital of the University of Pennsylvania.
[a] The nonneoplastic group includes scialadenitis, lypoma, and cysts.
[b] Benign neoplasms include Warthin tumor, pleomorphic adenoma, onocytoma, myoepithelioma, basal cell adenoma, and adenoma with myoepithelia/basal cell features.
[c] Malignant neoplasms include atypical, suspicious, and primary and metastatic lesions.
[d] $P > .05$ (not statistically significant).

TABLE 2. Cytohistologic Correlation of 709 Salivary Gland Fine-Needle Aspiration Cytology Cases at 2 Institutions

	R/P Histologic Follow-Up: No. of FNACs (Total No.)	
Findings	Benign	Malignant
No. (total no.), n = 709	354/201 (555)	72/82 (154)
Inadequate FNAC, n = 29	10/14 (24)	5/0 (5)
Benign, n = 564[a]	341/177 (518)	16/30 (46)
Nonneoplastic[b]	56/37 (93)	7/11 (18)
Benign neoplasm[b]	285/140 (425)	9/19 (28)
Atypical, n = 15[c]	0/7 (7)	0/8 (8)
Suspicious for malignancy, n = 28	3/3 (6)	9/13 (22)
Malignant[d]	0/0 (0)	38/35 (73)

Abbreviations: FNACs, fine-needle aspiration cytology cases; R/P, Catholic University "Agostino Gemelli" Hospital/Hospital of the University of Pennsylvania.
[a] The benign category includes nonneoplastic and benign-neoplasms (Warthin tumor, pleomorphic adenoma, onocytoma, myoepithelioma, basal cell adenoma, and adenoma with myoepithelia/basal cell features).
[b] $P < .05$ (statistically significant).
[c] $P > .05$ (not statistically significant).
[d] Malignant neoplasms include primary and metastatic lesions.

follow-up (426 from Catholic University and 283 from HUP). The clinicopathologic data, including cytologic diagnoses, are summarized in Table 1.

The cohort included 789 female and 940 male patients who ranged in age from 19 to 87 years (mean age, 47 years). There was no significant difference in the size of lesions among the diagnostic entities; the mean size ranged from 2.25 to 2.73 cm, as indicated in Table 1. Of the 1729 lesions, there were 1610 in the parotid gland, 71 in the submandibular gland, and 48 in the minor salivary glands (intraoral, palate, etc).

Surgical follow-up was available for 709 specimens, and the histologic diagnoses were rendered as benign in 78.3% and malignant in 21.7% (Table 2). The surgical pathology follow-up for specific diagnostic categories was available for 29 specimens diagnosed as inadequate, 564 diagnosed as benign (including 111 nonneoplastic and 453 benign neoplastic lesions) Fig. 1, 15 diagnosed as atypical, and 101 diagnosed as malignant (28 were suspicious for malignancy Fig. 2, and 73 were primary and metastatic neoplasms Fig. 3). Of the patients who had benign FNACs, 91.8% had a benign histologic outcome, and 94.1% of patients who had malignant FNACs had a malignancy diagnosed at follow-up.

The cytohistologic correlation of the 709 specimens that had surgical follow-up is depicted in Table 2. Specifically, the 111 nonneoplastic lesions with surgical pathology follow-up included 62 cystic lesions, 2 1ipomas, and 47 cases of chronic sialoadenitis. Overall, 93 of 111 (83.8%) nonneoplastic specimens resulted in a benign histologic diagnosis, and 18 (16.2%) resulted in a malignant histologic diagnosis. In total, 453 lesions that were diagnosed as benign neoplasm were surgically excised; of these, 425 (93.8%) had benign follow-up, and 28 (6.2%) had malignant follow-up. Surgical pathology follow-up was available for 28 lesions that were classified as suspicious for malignancy on FNAC; of these, 22 (78.6%) were diagnosed as malignant, and 6 (2.1%) were diagnosed as benign on histopathologic interpretation.

In total, 73 lesions that were diagnosed as malignant on FNAC were surgically excised; and all were identified as malignant. This group also included 18 secondary tumors

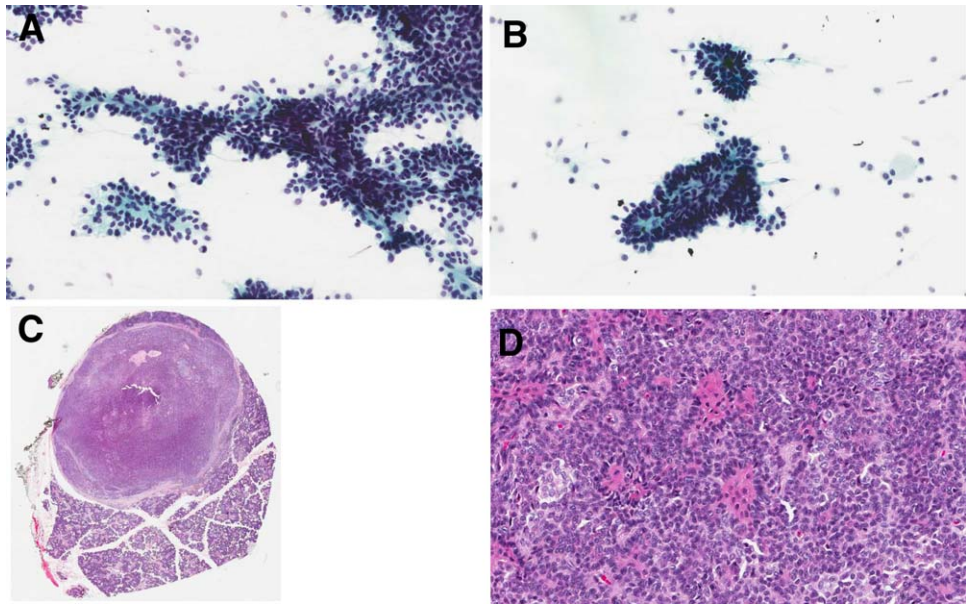

Figure 1. (A) A fine-needle aspiration cytology specimen reveals oval-to-spindle–shaped cells with intermixed stroma arranged in cohesive and branching tissue fragments (ThinPrep preparation, original magnification ×40). (B) A high-power image shows the lesion cells with evenly distributed, dense chromatin and lack of nuclear pleomorphism, findings diagnostic of pleomorphic adenoma (ThinPrep preparation, original magnification ×60). (C) Histologic follow-up reveals a circumscribed tumor confined within the salivary gland parenchyma (H&E-stained section, original magnification ×20). (D) Another high-power image confirms the diagnosis of cellular pleomorphic adenoma (H&E-stained section, original magnification × 40).

(24.7%). The most frequent malignant diagnosis was squamous cell carcinoma (25 lesions), followed by poorly differentiated carcinoma (17 lesions), malignant neoplasms not otherwise specified (NOS) (9 lesions), lymphoma (7 lesions), mucoepidermoid carcinoma (6 lesions), acinic cell carcinoma (5 lesions), and melanoma (4 lesions). Among the 25 squamous cell carcinomas, 6 (24%) presented as metastases from other head and neck primaries.

On the basis of histopathologic follow-up, there were 13 false-positive and 46 false-negative results (Table 3). Specifically, all false-positive specimens were initially diagnosed as either atypical or suspicious for malignancy. The most frequent instance of false-negative follow-up results were in lesions that were identified as lymphomas on surgical pathology follow-up (7 lesions); these were diagnosed as chronic sialoadenitis on FNAC. In addition, 15 lesions that were diagnosed as neoplasms NOS resulted in diagnoses of 7 low-grade mucoepidermoid carcinomas, 3 acinic cell carcinomas, 1 adenoid cystic carcinoma, 1 carcinoma expleomorphic adenoma, 1 salivary duct carcinoma, 1 adenocarcinoma NOS, and 1 metastatic carcinoma.

Performance measures were calculated after we excluded 29 specimens that were deemed inadequate/unsatisfactory for cytologic diagnosis. The analysis was performed with and without the inclusion of FNACs that were diagnosed as atypical in the malignant category. The first scenario resulted in 69.1% sensitivity, 97.6% specificity, 91.8% NPV, 88.8% PPV, and 91.3% diagnostic accuracy; and the second scenario produced 67.4% sensitivity, 98.9% specificity, 91.8% NPV, 94.1% PPV, and 92.2% diagnostic accuracy.

A comparative analysis between cases from the 2 institutions did not reveal any significant differences and strongly supported the diagnostic value of both conventional and LBC preparations. Table 4 describes the categories and risk of malignancy, and Table 5 suggests a proposed classification system based on the risk of malignancy.

DISCUSSION

The diagnostic role of FNAC in the evaluation of salivary lesions has been thoroughly evaluated and reported in the literature.[1–8] It would not be an overstatement to say that, despite the rarity of salivary lesions, the majority of nonneoplastic, benign, and most common malignant neoplasms are easily recognized and correctly diagnosed in cytologic specimens. However, it is also well recognized

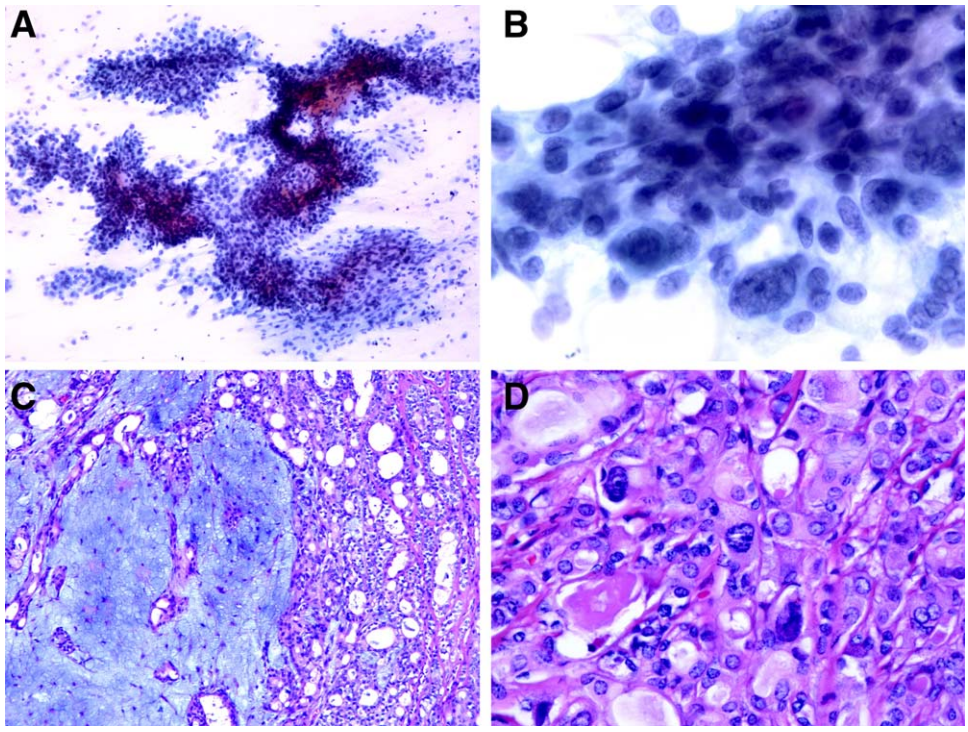

Figure 2. (A) Fine-needle aspiration cytology reveals a cellular specimen comprised of round-to-oval cells arranged in cohesive and branching tissue fragments (alcohol-fixed, Papanicolaou-stained smear, original magnification ×40). (B) A high-power image reveals the presence of randomly distributed cells with nuclear pleomorphism. This case was diagnosed as "atypical, malignant tumor cannot be excluded" (ThinPrep preparation, original magnification ×60). (C) Histologic follow-up showed a circumscribed tumor compatible with pleomorphic adenoma (H&E-stained section, original magnification ×20). (D) Another high-power image confirms the presence of the randomly distributed pleomorphic cells that were observed in the cytology specimen (H&E-stained section, original magnification ×60).

that even the evaluation of salivary gland FNAC specimens by experienced cytopathologists can lead to inconclusive diagnostic information, resulting in minimal influence on clinical and surgical management.[14,19,20] Most agree that this is because of the heterogeneous morphology of salivary gland lesions, mainly those comprised of epithelial and myoepithelial elements.[14,19] Tyagi and Dey offered an overall view of the causes of diagnostic challenges encountered with salivary gland FNAC; these included the heterogeneous nature of salivary gland neoplasms, resemblance to normal salivary gland elements, the presence of cystic components, overlapping cytologic features between benign and malignant lesions, and the presence of clear cells and oncocytic metaplasia.[17] For example, based on cytomorphology alone, it may not be possible to differentiate between myoepithelioma and myoepithelial carcinoma or between basal cell adenoma and carcinoma.[21,22]

In the current analysis of 1729 FNAC specimens from salivary gland lesions performed at 2 large institutions, 78.3% of specimens were benign lesions, and 21.7% were malignant, highlighting the established finding that the majority of salivary gland lesions are benign. The cytohistologic correlation was concordant in 91.8% of benign lesions and in 88.8% of malignant lesions, a value higher than that reported by some authors in the literature.[2,15] The risk of malignancy was 53.3% for 15 specimens diagnosed as "atypical," suggesting the clinical validity of this diagnostic category (Table 4). The statistical analysis indicated showed high specificity and diagnostic accuracy with and without inclusion of the 15 specimens that were diagnosed as atypical. This further proves the validity of salivary gland FNA as a reliable diagnostic procedure.

According to the literature, the rate of false-positive salivary gland FNAC results can range from 0% to 10%.[6,15,18] In our series, there were no false-positive results in specimens that were classified as malignant; however, 6 specimens (21.4%) that were diagnosed as "suspicious for malignancy" had benign histologic follow-up. These included Warthin tumor (2 specimens), sialoadenosis (1 specimen), PA (1 specimen), basal cell adenoma

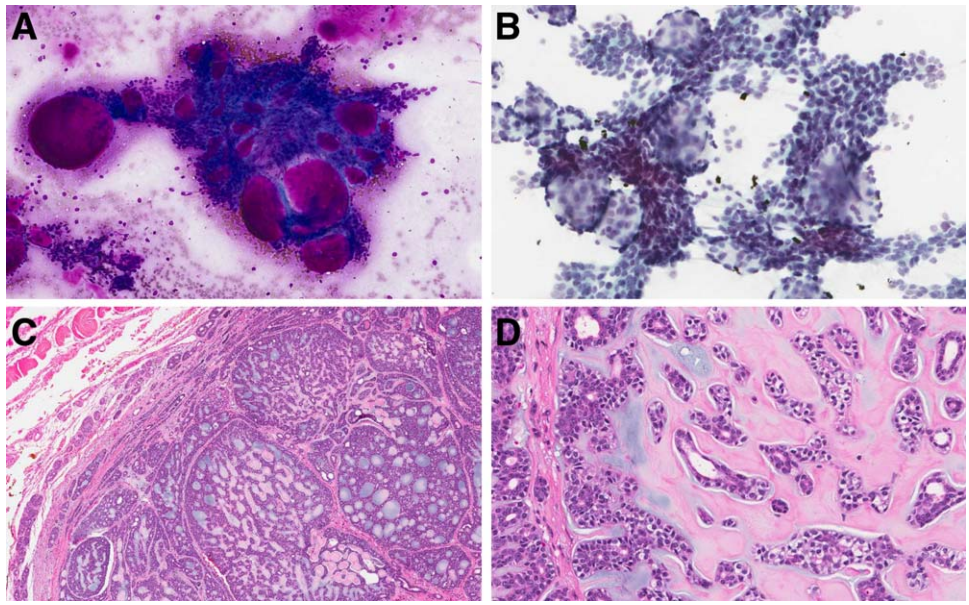

Figure 3. (A) Fine-needle aspiration cytology reveals diagnostic cytologic features of adenoid cystic carcinoma with acellular round-to-oval deposits of dense basement-type material surrounded by basaloid lesional cells (Diff-Quik–stained, air-dried smear preparation, original magnification ×40). (B) A corresponding ThinPrep preparation shows the presence of lesional basaloid cells and loss of dense basement membrane-type material (ThinPrep preparation, original magnification ×60). (C) Histologic follow-up reveals adenoid cystic carcinoma demonstrating tubular and cribriform patterns with hyaline and eosinophilic material and tumor infiltration at the periphery (H&E-stained section, original magnification ×20). (D) A high-power image reveals basaloid lesional cells and intervening hyalinized stroma (H&E-stained section, original magnification ×40).

TABLE 3. Cytohistologic Correlation of the 46 False-Negative and 13 False-Positive Fine-Needle Aspiration Cases[a]

Findings	Histologic Follow-Up (No. of Cases)
False-negative cases	
Chronic sialadenitis, n = 7	Malt lymphoma (1), non-Hodgkin lymphoma (6)
Cystic lesions, n = 11	Non-Hodgkin lymphoma (5), mucoepidermoid Ca (2), sarcoma (1), salivary duct Ca (1), epimyoepithelial Ca (1), metastasis (1)
Pleomorphic adenoma, n = 8	Epimyoepithelial Ca (4), adenoid cystic Ca (2), mucoepidermoid Ca (1), metastasis (1)
Neoplasm NOS, n = 15	Mucoepidermoid Ca (7), acinic cell Ca (3), adenoid cystic Ca (1), ex-PA Ca (1), salivary duct Ca (1), adenocarcinoma NOS (1), metastasis (1)
Oncocytoma, n = 1	Adenocarcinoma NOS (1)
Myoepithelioma, n = 1	Poorly differentiated Ca (1)
Basal cell/monomorphic adenoma, n = 2	Adenocarcinoma NOS (2)
Spindle cell neoplasm, n = 1	Sarcoma (1)
False-positive cases	
Atypical epithelial/Lymphoid/NOS, n = 7	Cysts (3), sialoadenosis (2), Warthin tumor (1), spindle cell neoplasm NOS (1)
Epithelial neoplasm suspicious for malignancy, n = 6	Warthin tumor (2), basal cell adenoma (2), sialoadenosis (1), pleomorphic adenoma (1), papillary lesion (1)

Abbreviations: Ca, carcinoma; ex-PA, expleomorphic adenoma; NOS, not otherwise specified.
[a] Twenty-nine inadequate cases were excluded from this correlation.

(1 specimen), and papillary lesion (1 specimen). Similar to the experience of other authors, PA was the most common benign FNAC neoplasm in the current series (37.7%), followed by Warthin tumor (15.2%). Although PAs accounted for the highest cytologic-histologic concordance, 8 specimens that were diagnosed as PA had a malignant follow-up, accounting for 17.4% of the false-negative rate; however, this was much lower compared with the 32% rate reported by the College of American Pathologists.[18] Nonetheless, these results underline the challenges in

TABLE 4. Combined Institutional Data Showing Salivary Gland Fine-Needle Aspiration Biopsy Diagnostic Categories, Surgical Follow-Up, and Risk of Malignancy

	FNAB Diagnostic Categories, No. of Cases (%)					
Variable	ND/Unsatisfactory	Nonneoplastic	Neoplasm	Atypical	Suspicious	Malignant
Total no. of FNABs, n = 1729	101 (6)	323 (19)	1074 (62)	25 (1)	49 (3)	157 (9)
Surgical FU, n = 709 (41%)						
Benign surgical FU, n=555	24	93	425	7	6	0
Malignant surgical FU, n = 154	5	18	28	8	22	73
Total (% surgery)	29 (29)	111 (34)	453 (42)	15 (60)	28 (57)	73 (46)
Risk of malignancy, %						
ROM	17	16	6	53	79	100
RO-HGM	7	1	3	13	46	58

Abbreviations: FNAB, fine-needle aspiration biopsy; FU, follow-up; ND, nondiagnostic; RO-HGM, risk of high-grade malignancy; ROM: risk of malignancy.

TABLE 5. Salivary Gland Fine-Needle Aspiration Diagnoses Based on the Risk of Malignancy

Diagnostic Category	Risk of Malignancy, %	Risk of High-Grade Epithelial Malignancy, %[a]
Nondiagnostic/unsatisfactory for interpretation	17	7
Nonneoplastic	16	1
Neoplasm	6	3
Atypical	53	13
Suspicious/malignant	94	54

[a] These calculations do not include cases that were diagnosed as lymphoma, metastatic carcinoma, or sarcoma on surgical follow-up.

differentiating PA in salivary gland FNAC specimens from specific malignant tumors, such as adenoid cystic carcinoma and epimyoepithelial carcinoma.[18]

In the current study, the use of different cytologic preparations at both institutions did not affect cytologic interpretation. Despite some reluctance to use LBC for salivary gland FNAC specimens (mainly ascribed to artifacts, cellular changes, and background alterations), various authors have reported no significant negative effect of LBC preparations on the cytologic interpretation.[23,24] Parfitt et al reported a comparative analysis of 98 salivary lesions that were processed using both conventional and LBC and concluded that these preparations are complementary to each other and can achieve higher diagnostic accuracy.[24] Rarick et al emphasized the value of LBC as the chosen method for evaluating salivary gland FNA specimens after only a short training period, specifically regarding the preparation-related artifacts.[23] Hipp et al, in their analysis of 369 salivary gland lesions processed with both LBC and conventional preparations, concluded that LBC rendered a lower amount of inadequate diagnoses and benign neoplasms.[25] In our experience, diagnostic concordance was achieved between LBC and conventional cytology preparations in all cases, thus attesting to the finding that good-to-excellent sensitivity and predictive value can be achieved for salivary gland FNAC specimens processed with both LBC and conventional cytologic preparations. Conversely, the use of Giemsa-stained smears is helpful in identifying stromal components. Because of this, many cytopathologists prefer to use a combination of smear and LBC for diagnosing salivary gland FNAC specimens.

Recently, Griffith et al proposed classifying salivary gland FNAC specimens based on key cytomorphologic features to achieve risk-based categorization. In their retrospective study, the FNAC specimens had been diagnosed and classified as unsatisfactory/nondiagnostic, benign, neoplasm of uncertain malignant potential, suspicious for malignancy, and malignant.[20] The overall risk of malignancy ranged from 2% for specimens classified as benign to 100% for those classified as positive for malignancy. We classified our specimens into 6 categories instead of 5: nondiagnostic/unsatisfactory, nonneoplastic, neoplasm, atypical, suspicious, and malignant; and the risk of malignancy ranged from 6.2% for benign lesions to 100% for malignant lesions (Tables 4 and 5). It is noteworthy that the risk of malignancy decreased from 100% to 94.1% when both suspicious and malignant categories were combined (Table 5). The statistical analysis, based on histopathologic correlation, revealed that outcomes for the diagnostic categories nonneoplastic, benign, and malignant (combined suspicious and malignant) were statistically significant. However, this was not the true for lesions in the diagnostic category "atypical," and we believe this was because of the limited number of specimens in this cohort.

The additional risk evaluation of salivary gland FNAC is accomplished by considering the rate of high-grade malignancy for each diagnostic category, as suggested by Griffith et al.[20] This knowledge may prove to be helpful for our clinical colleagues to manage patients with salivary gland lesions and determine the extent of surgical excision based on FNAC results. According to the suggestion of Griffith and colleagues, we divided the malignant histologic follow-up (excluding lesions diagnosed as lymphoma, metastatic carcinoma, and sarcoma in each category) into low-grade and high-grade primary salivary gland epithelial malignancy. On histologic follow-up in our study, the low-grade salivary gland epithelial malignancies were acinic cell carcinoma, basal cell adenocarcinoma, epithelial-myoepithelial carcinoma, low-grade and intermediate-grade mucoepidermoid carcinoma, and expleomorphic adenoma; and the high-grade epithelial tumors were adenoid cystic carcinoma, salivary duct carcinoma, poorly differentiated carcinoma, high-grade mucoepidermoid carcinoma, adenocarcinoma NOS, and metastatic squamous cell carcinoma. When we calculated the risk of high-grade epithelial malignancy of salivary gland origin, it ranged from 1% for nonneoplastic lesions to 58%, for lesions classified as malignant (Table 4). We believe that this categorization into low-grade and high-grade salivary gland neoplasms in FNAC specimens may be challenging because of inherent overlapping morphologic features and lesion heterogeneity (ie, high-grade lesions containing a foci of low-grade morphology). However, in most of these samples, a notification regarding the grade of a salivary gland neoplasm can be made based on the qualitative and quantitative assessment of cellular differentiation and pleomorphism.

The nondiagnostic rate in our study was 5.8%, with a 17.2% overall risk of malignancy and 7% risk for a high-grade epithelial malignancy. The nondiagnostic specimens in this series comprised of minimal amount of normal salivary gland tissue in the presence of a salivary gland lesion encountered by either physical or radiologic examination. To date, adequacy criteria for the evaluation of FNAC specimens of salivary gland lesions have not been established. On the basis of our review of the literature, most authors have used the presence or absence of lesion cells as enough to render a diagnosis without any quantitative qualifiers.[1–8,25] An adequacy criterion of $\geq$4 clusters of epithelial cells per high-power field has been used in some studies for the diagnosis of epithelial lesions of salivary gland origin; however, this will require validation by multiple independent studies in both LBC and conventional cytologic preparations.[20]

In our series, we did not perform immunocytochemistry or molecular analysis. Several studies have emphasized the role of ancillary techniques, including immunohistochemistry and molecular analysis, in enhancing the diagnostic accuracy of salivary gland lesions in histologic material. Some authors have used these studies in cytologic specimens with promising outcomes.[26–31] It has been demonstrated that malignant salivary tumors are driven by specific somatic mutations, including gene fusion, which can be studied by parallel RNA sequencing as well as whole-exome gene sequencing, even on cytologic material.[29,30] However, further evaluation of these ancillary techniques is needed in a large case cohort to ascertain their applicability in the diagnosis of salivary gland lesions in FNAC specimens.

In conclusion, our results attest to the finding that salivary gland FNAC is an effective diagnostic procedure for the evaluation of salivary gland lesions based on high sensitivity and specificity regardless of the cytologic preparation. Furthermore, it is feasible to classify FNAC specimens of salivary gland lesions into broader risk-based diagnostic categories for the effective clinical and surgical management of patients.

FUNDING SUPPORT

No specific funding was disclosed.

CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Esther Diana Rossi: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing–original draft, visualization, supervision, project administration. **Lawrence Q. Wong**: Validation, formal analysis, investigation, resources, data curation, writing–original draft, writing–review and editing, visualization. **Tommaso Bizzarro**: Methodology, software, formal analysis, data curation. **Gianluigi Petrone**: Visualization. **Antonio Mule**: Visualization. **Guido Fadda**: Writing–review and editing. **Zubair Baloch**: Conceptualization, methodology, formal analysis, writing–original draft, writing–review and editing.

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