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The diagnostic accuracy of pleomorphic adenoma in fine needle aspiration: Features leading to false positive and false negative diagnoses and the utility of cell blocks

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Abstract

Objective: Fine needle aspiration (FNA) is a well-established tool in preoperative diagnosis of salivary gland lesions with diagnostic accuracy of 90%. Pleomorphic adenoma (PA) is the most common salivary gland tumor comprising 45%–74% of all salivary gland tumors with FNA diagnostic accuracy of 89.5%–96.2%. The aim of the present study was to determine and analyze potential cytomorphological pitfalls and evaluate the diagnostic accuracy in FNA diagnosis of PA.

Methods: Salivary gland specimens with both cytological and histological diagnoses were searched over a 10-year-period (2009–2018) from a laboratory information system of Pathology Department, Fimlab Laboratories, Tampere and matched to determine concordant and discordant PA cases. Sufficient material in histological and cytological sample was found in 401 cases. In 218 cases (54.4%) diagnosis was true-negative PA, in 169 cases (42.1%) diagnosis was true-positive PA and there were 14 discordant cases: 4 false-positive cases and 10 false-negative cases. False-negative cases were reclassified and subgrouped according to The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC).

Results: Cytomorphologically, cell type predominance was more often myoepithelial in true-positive cases (65%) and epithelial both in false-negative (70%, $p = .007$) and false-positive cases (75%, $p = .027$). Well-formed ducts were present in cytology in all true-positive cases ($p < .001$). Only 10% of true-positive cases did not show any matrix in cytology ($p < .001$). Nuclear changes were common in false-negative cases (80%, $p = .002$) and false-positive cases (75%, $p = .003$). Beneficial cell block (CB) was more common in true-positive cases (85%) than in false-negative cases (50%, $p = .041$) or in false-positive cases (50%, $p = .116$) and a lack of beneficial CB led more often to a false diagnosis (70% false diagnosis without beneficial CB versus 29% false diagnosis with beneficial CB).

Conclusion: The present study showed diagnostic accuracy of 96.5% for FNA in PA diagnosis. Sensitivity, specificity, positive predictive value and negative predictive value were 94.4%, 98.2%, 97.7%, and 95.6%, respectively. The benefit of CBs was more evident in true-positive cases (85%).

KEYWORDS
FNA, pleomorphic adenoma, salivary glands, The Milan System for Reporting Salivary Gland Cytopathology

1 | INTRODUCTION

Fine needle aspiration (FNA) is a commonly used and well-established minimally invasive cost-effective tool for determining preoperative diagnosis in salivary gland lesions.[1–6] Recent meta-analysis showed overall lower sensitivity of 80% and higher specificity of 97% in salivary gland FNA.[4] Pleomorphic adenoma (PA) is the most common salivary gland tumor comprising 45%–74% of all salivary gland tumors.[6,7] The diagnostic accuracy of FNA has been shown to be 89.5%–96.2% in PA diagnostics.[8] However, cytological diagnosis of PA may cause diagnostic dilemmas due to the overlapping cytological features and diversity of cytomorphological characteristics.[9,10] Regarding cytological diagnosis of PA, the most common false-negative diagnoses reported in the literature were adenoid cystic carcinoma,[6,8,11,12] mucoepidermoid carcinoma[5,6,12,13] and cystic lesions.[5,6,11,12] In comparison, the most common reported false-positive diagnoses were adenoid cystic carcinoma,[6,12,14–16] mucoepidermoid carcinoma,[14–16] monomorphic adenoma,[6,16] myoepithelioma,[6,12] and carcinoma ex pleomorphic adenoma.[16,17] According to a recent study, the cell block (CB) and ancillary tests improved the diagnostics in 100% of benign neoplasm cases and in 98.3% of malignant cases.[18]

The aim of the present study was to determine and analyze potential cytomorphological pitfalls and evaluate the diagnostic accuracy in FNA diagnosis of PA in a university based tertiary care center over a 10-year-period. The role of CBs and immunohistochemistry (IHC) was also evaluated.

2 | MATERIALS AND METHODS

A laboratory information system at Pathology Department, Fimlab Laboratories, Tampere was searched over a 10-year-period (2009–2018) for specimens from parotid glands and submandibular glands with both cytological diagnosis and histological follow-up diagnosis. Cases with cytologically insufficient material were excluded to determine diagnostic accuracy of PA. In addition, sex, age, topography, and size of lesion for each case were recorded.

The fine needle aspirations (FNAs) were performed by radiologists with 22G needles under ultrasound control. The specimens were alcohol-fixed, cytospun, and stained with Papanicolaou stain. The rest of the alcohol-fixed material was used for CB preparation. Various methods were used to prepare CBs[19]: plasma-thrombin method, collection of visible tissue fragments, in-house method[20] and commercial Shandon CB method.[19]

For cases with either cytological or histological PA diagnosis, cytological and histological diagnoses were matched to determine concordant and discordant cases. Histological follow-up diagnoses were used as a golden standard to divide discordant cases into false-positive and false-negative categories. False-positive category included cases with cytological PA diagnosis and histological diagnosis other than PA. False-negative category included cases with cytological diagnosis other than PA and histological PA diagnosis. Cases in false-negative category were evaluated and subgrouped according to The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC). Cases with both cytological and histological PA diagnoses were classified as true-positive and cases with neither cytological nor histological PA diagnoses were classified as true-negative. In one patient two FNAs were performed and only the prior FNA was included in the analysis.

All cases with false-positive or false-negative cytological PA diagnoses were re-evaluated and compared with 20 randomly selected true-positive cases. All cytological and histological slides including CBs and immunohistochemical sections were re-evaluated.

p-values were calculated for cytological diagnoses and other characteristics by IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, NY) using the Cramer's *V*. Statistical measures were calculated for cytological PA diagnosis.

Pirkanmaa Hospital District Ethical committee approved the study (R17174). All procedures were performed in accordance with Helsinki Declaration (1975, revised 1983). Informed consent of each individual was not requested.

3 | RESULTS

A total of 401 patients with cytologically sufficient specimen had follow-up histological diagnosis during the study period. In 179 (45%) cases the histological diagnosis was PA. Tumor location was parotid gland in 160 (89%) cases and submandibular gland in 19 (11%) cases. There were 64 (36%) males and 115 (64%) females, mean age was 53 ± 1.3 years (range 14–91) and mean tumor size (reported in 135/179 cases) was 2.0 ± 0.1 cm (range 0.8–5.0 cm). There were 218 (54.4%) true-negative cases with neither cytological nor histological PA diagnoses. Either cytological or histological PA diagnosis was given in 183 (45.6%) cases. There were 169 (92.3%) concordant cases with either definitive or descriptive cytological PA diagnosis that were histologically confirmed PAs (Table 1; Figure 1A–D).

Statistical analysis showed diagnostic accuracy of 96.5% in FNA PA diagnosis. Sensitivity, specificity, positive predictive value, and negative predictive value were 94.4%, 98.2%, 97.7%, and 95.6%, respectively (Table 1).

Altogether there were 14 (7.7%) discordant cases: 4 (2.2%) false-positive cases and 10 (5.5%) false-negative cases. False-positive cases included following histological diagnoses: epithelial-myoepithelial carcinoma ($n = 2$), mucoepidermoid carcinoma, low grade ($n = 1$) and carcinoma ex pleomorphic adenoma ($n = 1$) (Figure 1I–P). False-negative cases were subgrouped according to the MSRSGC. Suspicious for

TABLE 1 Study cohort characteristics and diagnostic accuracy of pleomorpic adenoma diagnoses.

Diagnostic accuracy 96.5%	Sensitivity 94.4%	Specificity 98.2%	PPV 97.7%	NPV 95.6%
401	Cases with sufficient material in histological and cytological sample			
	218 (54.4%)	True-negative PA		
	169 (42.1%)	True-positive PA		
	4 (1.0%)	False-positive PA		
		2	Histologically epithelial–myoepithelial carcinoma	
		1	Histologically mucoepidermoid carcinoma, low grade	
		1	Histologically carcinoma ex pleomorphic adenoma	
	10 (2.5%)	False-negative PA		
		6	MSRSGC reclassification as suspicious for malignancy	
			6	Cytologically atypia, suspicious for malignancy
		4	MSRSGC reclassification as SUMP	
			1	Cytologically neoplasm, uncertain whether benign or malignant
			1	Cytologically neoplasm, NOS
			1	Cytologically basaloid tumor
			1	Cytologically oncocytic tumor

Abbreviations: NOS, not otherwise specified; NPV, negative predictive value; PA, pleomorphic adenoma; PPV, positive predictive value; SUMP, Neoplasm of Uncertain Malignant Potential.

Malignancy subgroup included following cytological diagnoses: atypia, suspicious for malignancy ($n = 6$) and SUMP subgroup included following cytological diagnoses: neoplasm, uncertain whether benign or malignant ($n = 1$), neoplasm, benign ($n = 1$), basaloid tumor ($n = 1$) and oncocytic tumor ($n = 1$) (Figure 1E–H).

Different characteristics of 20 randomly selected true-positive cases compared to false-negative and false-positive cases are presented in Table 2. Beneficial CB was more common in true-positive cases (85%, 17/20) than in false-negative cases (50% 5/10, $p = .041$) or in false-positive cases (50%, 2/4, $p = .116$) and a lack of beneficial CB led more often to a false diagnosis (70%, 7/10 false diagnosis without beneficial CB versus 29%, 7/24 false diagnosis with beneficial CB). IHC was performed only in 15% (3/20) of true-positive cases, but in 50% (5/10, $p = .041$) of false-negative cases and 25% (1/4) of false-positive cases (Table 3).

Selected cytomorphological features with significant differences are illustrated in Figures 1 and 2. Cell type predominance was more often myoepithelial in true-positive cases (65%, 13/20) and epithelial both in false-negative cases (70%, 7/10, $p = .007$) and in false-positive cases (75%, 3/4, $p = .027$). Well-formed ducts were present in cytology in all true-positive cases (20/20) and in 80% (8/10) of false-negative cases whereas well-formed ducts were present in cytology only in 25% (1/4) in false-positive cases ($p < .001$). Only 10% (2/20) of true-positive cases did not show any matrix in cytology whereas 70% (7/10) of false-negative cases did not show any matrix in cytology ($p < .001$). Nuclear combined changes (nuclear atypia, nuclear pleomorphism, multilobulated nuclei, coarse chromatin or nuclear crowding) were rare in true-positive cases (10%, 2/20) and common in false-negative cases (80%, 8/10, $p < .001$) and false-positive cases (75%, 3/4, $p = .003$). Metaplasia was slightly more common in discordant cases than in true-positive cases (14% vs. 5%), but the difference was not significant.

When false-positive carcinoma entities were separately evaluated, true-positive PA cases were characterized by myoepithelial predominance, presence of well-formed ducts and myxoid matrix that were present naturally in carcinoma ex PA, but not in other malignancies. Nuclear atypia was more common both in false-positive and false-negative cases (Table 4).

4 | DISCUSSION

PA is the most common salivary gland tumor comprising 45%–74% of all salivary gland tumors[6,7] and its diagnostic accuracy in FNA has been shown to be 89.5%–96.2%.[8] After implementation of the MSRSGC, international, multi-institutional study revealed 95.1% diagnostic accuracy for PA.[21] In the present study, a 10-year-period diagnostic accuracy of PA diagnosis in tertiary care center was 96.5%. Sensitivity was 94.4%, specificity 98.2%, positive predictive value 97.7%, and negative predictive value 95.6%, respectively. Altogether there were 14 (7.7%) discordant cases consisting of 4 (2.2%) false-positive cases and 10 (5.5%) false-negative cases. In a Spanish series of cyto-histologically correlated 175 PA cases with 7.1% false-negative rate: 9 cases were false-positive and 12 cases false-negative.[6] In a later analysis from same group, concordant cases increased to 91.2% and false-negative rate diminished to 4.5%.[12] In

a literature analysis, false-positive cases ranged from 1.3% to 4.7%.[11] Retrospective analysis of the College of American Pathologists Non-gynecologic Cytology Program's data showed PA false-positive rate of 8%.[15] In that respect, our data are within the rates of other studies.

PA is known for architectural and cytomorphological heterogeneity, proportion of its components is variable, and the appearance of epithelial and myoepithelial cells is varying. In the literature various lesions and cytomorphological features causing diagnostic pitfalls and false negativity or false positivity in PA diagnostic work-up has been described. Cellularity, mucoid stroma, nuclear atypia, oncocytic metaplasia and lack of chondromyxoid stroma were encountered in discordant cases in a large series of 412 cases.[12] Viguer et al.[6] series summarized following diagnostic cytomorphological pitfalls in PA: cellularity with epithelial atypia, epithelial predominance, and cystic pattern. In our series, epithelial predominance, low myoepithelial cellularity and nuclear changes were more common in both false-negative and false-positive cases than in true-positive cases. Lack of well-formed ducts was featured in false-positive cases. On the other hand, lack of matrix was present in false-negative cases. Myoepithelial predominance was featured in true-positive cases with the exception of epithelioid myoepithelial cells present in false-negative cases. Heterogeneity is common in PA, but abundancy of atypical cells and necrosis are worrisome features against PA diagnosis.[12] In the line, nuclear atypia was also common in discordant cases in the present series. Metaplasia is widely reported as a diagnostic pitfall in the

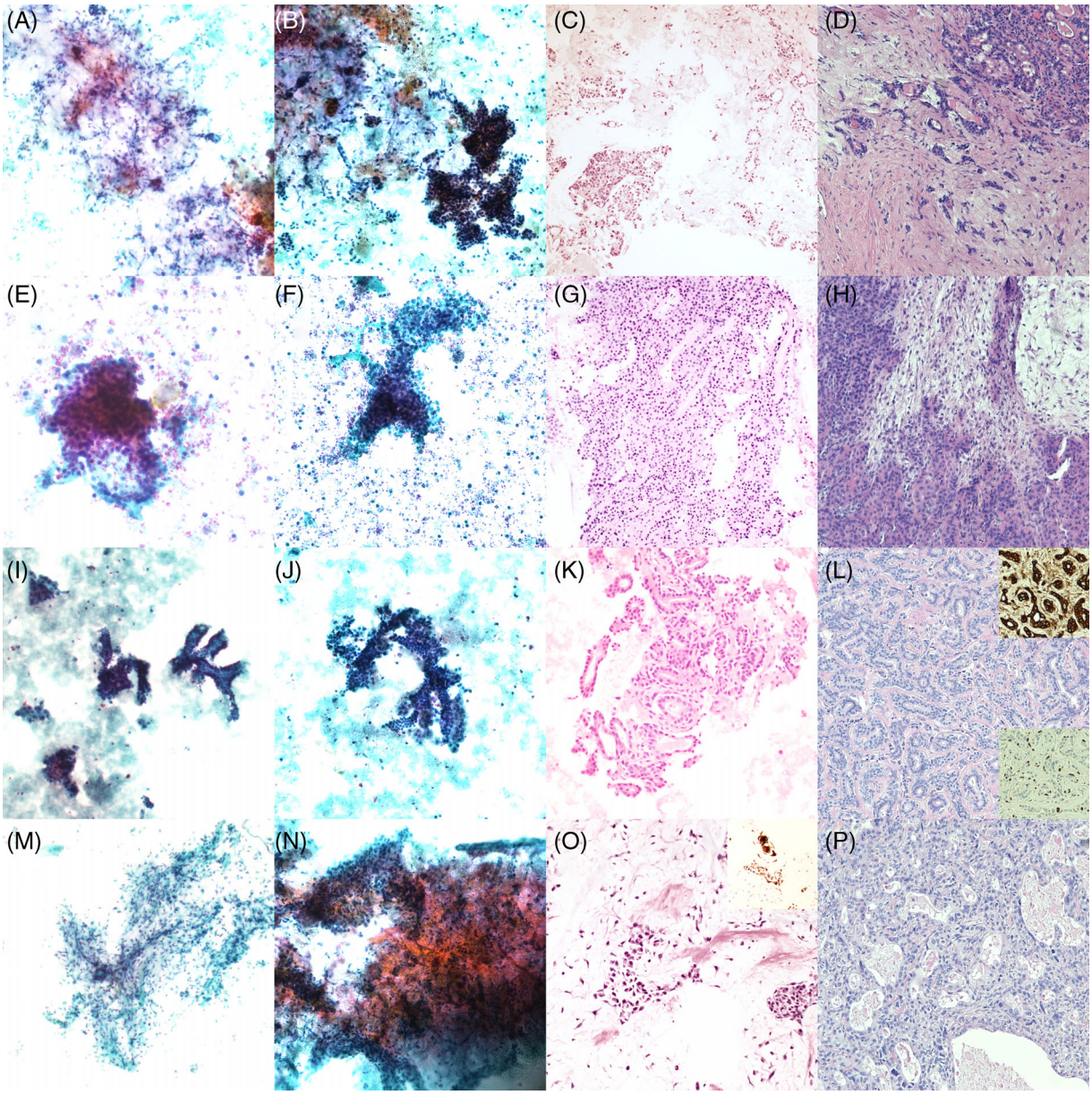

FIGURE 1 Legend on next page.

literature,[6,11,12] but was not a major problem in our cases. Cystic lesions were found problematic in some series,[12,22] but not in our discordant cases. Features related to discordant cases are listed in Table 5.

In majority of our discordant cases, cytomorphological features were misinterpreted. Epithelial predominance led both to false-positive and false-negative cases as well as nuclear atypia was both over-interpreted and under-interpreted. Epithelial predominant cases were easily misdiagnosed. In carcinoma ex PA malignant component is often focal. The reasons for FNA misdiagnosis of carcinoma ex PA can be differently rooted: (1) sampling error when only PA component is targeted by needle, (2) misinterpretation of focal atypia as a part of normal cytological diversity[12] as it happened in our case (Figure 1M–P). Due to misinterpretation, we grouped the case as false-positive. In case of sampling error, the cases may be classified as true-positive after slide revision if no atypia is present. Literature on epithelial-myoepithelial carcinoma cytomorphology is sparse.[23,24] Common features of PA and epithelial-myoepithelial carcinoma are biphasic cell population with epithelial and myoepithelial component and mild or no atypia, that was misinterpreted in our two cases. Secreted acellular laminated basal membrane-like stroma may also be challenging in these cases. Evaluation of stromal component in Papanicolaou-stained specimens may be tricky and additional Giemsa-based staining could be beneficial. In one series, one case of epithelial–myoepithelial carcinoma was placed in MSRSGC AUS category and majority of cases in SUMP category showing diagnostic challenges.[25]

PA cytology samples represent well most of the PA histological features namely overall cellularity and the proportion of epithelial, myoepithelial and mesenchymal component with exception of chondroid metaplasia being underrepresented in cytology.[9] Of note, some unusual and uncommon morphological features as bizarre nuclei, necrosis and metaplasia can cause diagnostic problems also in histological specimens.[26]

International, multi-institutional study showed that 95.1% PA cases were placed in benign neoplasm MSRSGC diagnostic category and only 5.5% in SUMP category. The most common false-negative benign neoplasm was basal cell adenoma (0.9%) and false-negative malignant neoplasm was carcinoma ex PA (36%) and adenoid cystic carcinoma (28%).[21] Memorial Sloan Kettering study showed 17.2% of PA placed in SUMP category.[25] Indian MSRSGC institutional series showed PA cases also in non-diagnostic and suspicious for malignancy categories with majority of cases in benign neoplasm category.[27] In our institutional MSRSGC series, 71% of PA cases were categorized as benign neoplasm, 23% as SUMP and 6% as AUS.[28] Cellular PAs with nuclear atypia and lack of matrix are naturally diagnosed in SUMP category. Low cellularity and cystic tumors lead to AUS category. Unified reporting system increases agreement and lessens inter-observer variability.

There is sparse literature on CB role in salivary gland tumor diagnostic work-up. In the present study beneficial CB was more common in true-positive cases (85%) than in false-negative (50%) and false-positive cases (50%) with a statistical significance. In the line, a lack of beneficial CB led more often to a false diagnosis (70% vs. 29% false diagnosis with beneficial CB). In our previous study, non-contributory CBs also resulted more often in a false-negative diagnosis (25%) than

FIGURE 1 (A) A true-positive case with cytological and histological diagnosis of pleomorphic adenoma in right parotid gland of a 38-year-old male. Cytological specimen showed rich myxomatous stroma with flowing spindle shaped myoepithelial cells and ductal structures representing epithelial component. Papanicolaou stain, magnification 100×. (B) More cellular area with prominent ductal and trabecular structures. Same case as (A). Papanicolaou stain, magnification 100×. (C) Cell block of same case as (A and B). Myxomatous background stroma with myoepithelial cells and ducts in-between. Hematoxylin–eosin, magnification 100×. (D) Surgical specimen revealed pleomorphic adenoma with ductal epithelium and variably thick stroma, flowing myoepithelial cells and fibrosis. Same case as (A, B and C). Hematoxylin–eosin, magnification 100×. (E) A false-negative case was originally diagnosed as oncocytic tumor in FNA of right parotid gland in a 67-year-old male. Oncocytic cell fragment and dispersed oncocytes in cytological specimen. Papanicolaou stain, magnification 100×. (F) 3-D tissue branching fragment formed by oncocytes. Same case as (E). Papanicolaou stain, magnification 100×. (G) Cell block of case (E and F) showed large oncocytic cell fragment that led to false-negative diagnosis. Oncocytic metaplasia is encountered as pitfall in pleomorphic adenoma diagnostics. Hematoxylin–eosin, magnification 100×. (H) Surgical specimen of pleomorphic adenoma showed large area of oncocytic metaplasia. Same case as (E, F, and G). Hematoxylin–eosin, magnification 100×. (I) A false-positive case of left parotid gland tumor in a 55-year-old male revealed biphasic pattern with myoepithelial and epithelial component, but no stroma. Original cytological diagnosis was pleomorphic adenoma, but histology revealed epithelial-myoepithelial carcinoma. Several trabecular biphasic fragments are present. Papanicolaou stain, magnification 100×. (J) Larger fragment with biphasic cell population. Note the absence of stroma. Background is hemorrhagic. Same case as (I). Papanicolaou stain, magnification 100×. (K) Cell block of (I and J) case showed also biphasic pattern of myoepithelial and epithelial component, but no stroma was found in the specimen. Hematoxylin–eosin, magnification 200×. (L) Surgical specimen of case (I, J and K). There is typical biphasic population and thick basement membrane-like stroma in epithelial–myoepithelial carcinoma. Hematoxylin–eosin, magnification 100×. Upper inset: Cytokeratin 7 IHC was performed in surgical specimen of (L) case resulting in positivity in ductal epithelial cells and myoepithelial cells with different intensity. Cytokeratin 7, magnification 100×. Lower inset: p63 IHC in a larger fragment of myoepithelial cell population in the surgical specimen showed nuclear positivity in myoepithelial cells. P63, magnification 100×. (M) A false-positive case of left parotid gland tumor in a 38-year-old male diagnosed cytologically as pleomorphic adenoma was carcinoma ex PA in histology. There were largely cytomorphological features of typical pleomorphic adenoma with only a limited area with mild nuclear atypia, that was interpreted as cellular diversity. Papanicolaou stain, magnification 100×. (N) Same case as (M). Specimen with mainly cytomorphological features of typical pleomorphic adenoma and a limited area of mild nuclear atypia. Papanicolaou stain, magnification 100×. (O) Cell block of (M and N) case showed mainly stromal component with limited cellularity. Nevertheless, mild nuclear size and shape variability is detectable. Hematoxylin–eosin, magnification 200×. (P) Surgical resection of the same case as shown in (M–O), revealed carcinoma ex PA histology. Moderate nuclear and architectural atypia in carcinoma component. Identical atypical cells were seen in cytological specimen. Hematoxylin–eosin, magnification 100×. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Summary of clinical and morphological features.

Feature	TP control group ($n = 20$)	FN all ($n = 10$)		FN SUMP ($n = 4$)		FN suspicious for malignancy ($n = 6$)		FP ($n = 4$)	
Sex (M/F)	(20%/80%)	(30%/70%)		(50%/50%)		(17%/83%)		(50%/50%)	
Age (years) ± SD (range)	49.3 ± 18.9 (17–79)	59.2 ± 16.3 (32–80)		60.5 ± 4.4 (57–59)		56.7 ± 21.4 (32–80)		59.7 ± 20.3 (38–87)	
Parotid gland	18/20 (90%)	7/10 (70%)		4/4 (100%)		3/6 (50%)		4/4 (100%)	
Submandibular gland	2/20 (10%)	3/10 (30%)		0/4 (0%)		3/6 (50%)		0/4 (0%)	
Cytology to histology timeframe	5.2 months	4.7 months		6.9 months		3.2 months		5.2 months	
Tumor size (cm) ± SD (range)	1.8 ± 0.57 (0.9–2.8)	5.3 ± 0.53 (0.8–2.0)		1.4 ± 0.85 (0.8–2.0)		1.2 ± 0.21 (1.0–1.3)		2.2 ± 0.42 (1.9–2.5)	
Histology cellular subtype	4 (20%)	8 (80%)	$p = .002$	4 (100%)	$p = .002$	4 (67%)	$p = .030$	4 (100%)	$p = .002$
Histology geographical heterogeneity	4 (20%)	6 (60%)	$p = .028$	1 (25%)		5 (83%)	$p = .004$	1 (25%)	
Cell block performed	19 (95%)	9 (90%)		3 (75%)		6 (100%)		3 (75%)	
Beneficial cell block	17 (85%)	5 (50%)	$p = .041$	2 (50%)		3 (50%)		2 (50%)	
IHC performed	3 (15%)	5 (50%)	$p = .041$	2 (50%)		3 (50%)		1 (25%)	
Matrix predominance	3 (15%)	1 (10%)		1 (25%)		0 (0%)		0 (0%)	
Epithelial predominance	4 (20%)	7 (70%)	$p = .007$	3 (75%)	$p = .027$	4 (67%)	$p = .030$	3 (75%)	$p = .027$
Myoepithelial predominance	13 (65%)	2 (20%)	$p = .020$	0 (0%)	$p = .017$	2 (33%)		1 (25%)	
Epithelial > myoepithelial	4 (20%)	7 (70%)	$p = .007$	3 (75%)	$p = .027$	4 (67%)	$p = .030$	3 (75%)	$p = .027$
Overall cellularity 1–3, avg.	2.3	2.0		2.0		2.0		2.3	
Plasmacytoid myoepithelial cells	13 (65%)	3 (30%)		0 (0%)	$p = .017$	3 (50%)		2 (50%)	
Spindle myoepithelial cells	14 (70%)	5 (50%)		2 (50%)		3 (50%)		1 (25%)	
Epithelioid myoepithelial cells	1 (5%)	3 (30%)		3 (75%)	$p < .001$	0 (0%)		0 (0%)	
Polygonal myoepithelial cells	2 (10%)	2 (20%)		0 (0%)		2 (33%)		1 (25%)	
Myoepithelial cellularity (0–3, avg.)	2.5	1.6		1.5		1.7		1.3	
Ductal cells	20 (100%)	8 (80%)	$p = .038$	3 (75%)	$p = .022$	5 (83%)	$p = .063$	1 (25%)	$p < .001$
Invidually dispersed cells	9 (45%)	7 (70%)		4 (100%)	$p = .044$	3 (50%)		1 (25%)	
Arrangement in small clusters	20 (100%)	6 (60%)	$p = .002$	2 (50%)	$p < .001$	4 (67%)	$p = .007$	3 (75%)	$p = .022$
Arrangement in large groups	11 (55%)	6 (60%)		3 (75%)		3 (50%)		2 (50%)	
Mucoid matrix	8 (40%)	0 (0%)	$p = .020$	0 (0%)		0 (0%)		0 (0%)	
Myxoid matrix	16 (80%)	2 (20%)	$p = .002$	1 (25%)	$p = .027$	1 (17%)	$p = .004$	2 (50%)	
Myxochondroid matrix	1 (5%)	0 (0%)		0 (0%)		0 (0%)		0 (0%)	
Hyaline matrix	0 (0%)	1 (10%)		0 (0%)		1 (17%)		1 (25%)	$p = .022$
No matrix	2 (10%)	7 (70%)	$p < .001$	3 (75%)	$p = .003$	4 (67%)	$p = .004$	1 (25%)	

TABLE 2 (Continued)

Feature	TP control group ($n = 20$)	FN all ($n = 10$)		FN SUMP ($n = 4$)		FN suspicious for malignancy ($n = 6$)		FP ($n = 4$)	
Hemorrhagic background	2 (10%)	2 (20%)		1 (25%)		1 (17%)		1 (25%)	
Squamous metaplasia	1 (5%)	0 (0%)		0 (0%)		0 (0%)		0 (0%)	
Oncocytic metaplasia	1 (5%)	1 (10%)		1 (25%)		0 (0%)		1 (25%)	
No metaplasia	19 (95%)	9 (90%)		3 (75%)		6 (100%)		3 (75%)	
Nuclear atypia	1 (5%)	5 (50%)	$p = .004$	1 (25%)		4 (67%)	$p < .001$	2 (50%)	$p = .013$
Nuclear pleomorphism	2 (10%)	3 (30%)		2 (50%)	$p = .050$	1 (17%)		3 (75%)	$p = .003$
Bilobed/multiobated nuclei	0 (0%)	1 (10%)		1 (25%)	$p = .022$	0 (0%)		0 (0%)	
Coarse chromatin	0 (0%)	6 (60%)	$p < .001$	1 (25%)	$p = .022$	5 (83%)	$p < .001$	2 (50%)	$p < .001$
Nuclear crowding	0 (0%)	1 (10%)		0 (0%)		1 (17%)		0 (0%)	
Any nuclear changes	2 (10%)	8 (80%)	$p < .001$	3 (75%)	$p = .003$	5 (83%)	$p < .001$	3 (75%)	$p = .003$
Epithelial predominance & nuclear changes	0 (0%)	6 (60%)	$p < .001$	3 (75%)	$p < .001$	3 (50%)	$p < .001$	3 (75%)	$p < .001$
Epithelial predominance & no matrix	0 (0%)	5 (50%)	$p < .001$	3 (75%)	$p < .001$	2 (33%)	$p = .007$	1 (25%)	$p = .022$
Epithelial predominance & no ductal cells	0 (0%)	2 (20%)		1 (25%)		1 (17%)		3 (75%)	$p < .001$

Note: *p*-values by SPSS & Cramer's *V*.

Abbreviations: avg., average; FN, false-negative; FP, false-positive; IHC, immunohistochemistry; SUMP, Neoplasm of Uncertain Malignant Potential; TP, true-positive.

TABLE 3 Summary of cell blocks and ancillary tests performed.

Diagnosis	No CB	Insufficient CB	Beneficial CB	IHC performed
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA		1		
TP: PA			1	
TP: PA			1	MIB-1, CK7, SMA, EMA, GFAP, MAMMAGL, AR, S-100
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	
TP: PA			1	MIB-1
TP: PA			1	CK7, CKPAN, Ki-67, CALPONIN
TP: PA		1		
TP: PA	1			
FN, SUMP: Neoplasm, uncertain whether benign or malignant		1		
FN, SUMP: Neoplasm, NOS	1			
FN, SUMP: Basaloid tumor			1	MIB-1
FN, SUMP: Oncocytic tumor			1	MIB-1, CK5/6, P63, CK7, MITOCH
FN, SM: Atypia, suspicious for malignancy			1	
FN, SM: Atypia, suspicious for malignancy			1	MIB-1, CKPAN, SMA, EMA
FN, SM: Atypia, suspicious for malignancy		1		P63, CK7, CKPAN, SMA, EMA, GFAP, S-100, CALPONIN, CD10, CD20, CD138
FN, SM: Atypia, suspicious for malignancy		1		
FN, SM: Atypia, suspicious for malignancy			1	MIB-1, CKPAN, SMA
FN, SM: Atypia, suspicious for malignancy		1		
FP: Epithelial–myoepithelial carcinoma	1			
FP: Mucoepidermoid carcinoma, low grade		1		
FP: Epithelial–myoepithelial carcinoma			1	
FP: Carcinoma ex pleomorphic adenoma			1	P63, CK7, SMA, EMA, S-100

Abbreviations: AR, androgen receptor; CB, cell block; CK5/6, cytokeratin 5/6; CK7, cytokeratin 7; CKPAN, pan-cytokeratin; EMA, epithelial membrane antigen; FN, false-negative; FP, false-positive; GFAP, glial fibrillary acidic protein; IHC, immunohistochemistry; MAMMAGL, mammaglobin; MITOCH, mitochondrial marker; PA, pleomorphic adenoma; SM, suspicious for malignancy; SMA, actin, alpha smooth muscle; SUMP, Neoplasm of Uncertain Malignant Potential; TP, true-positive.

a true-negative diagnosis (10%) with a statistical significance.[29] Interestingly, Behaeghe et al.[30] retrospectively analyzed 359 salivary gland samples processed only as a Cellient CB in view of the MSRSGC with an overall accuracy of 92.9%.

The need of ancillary techniques in salivary gland pre-operative diagnosis is increasing with the growing amount of known genetic mutations and rearrangements in salivary gland tumors.[31] Main molecular events in PA such as translocations of *Pleomorphic adenoma*

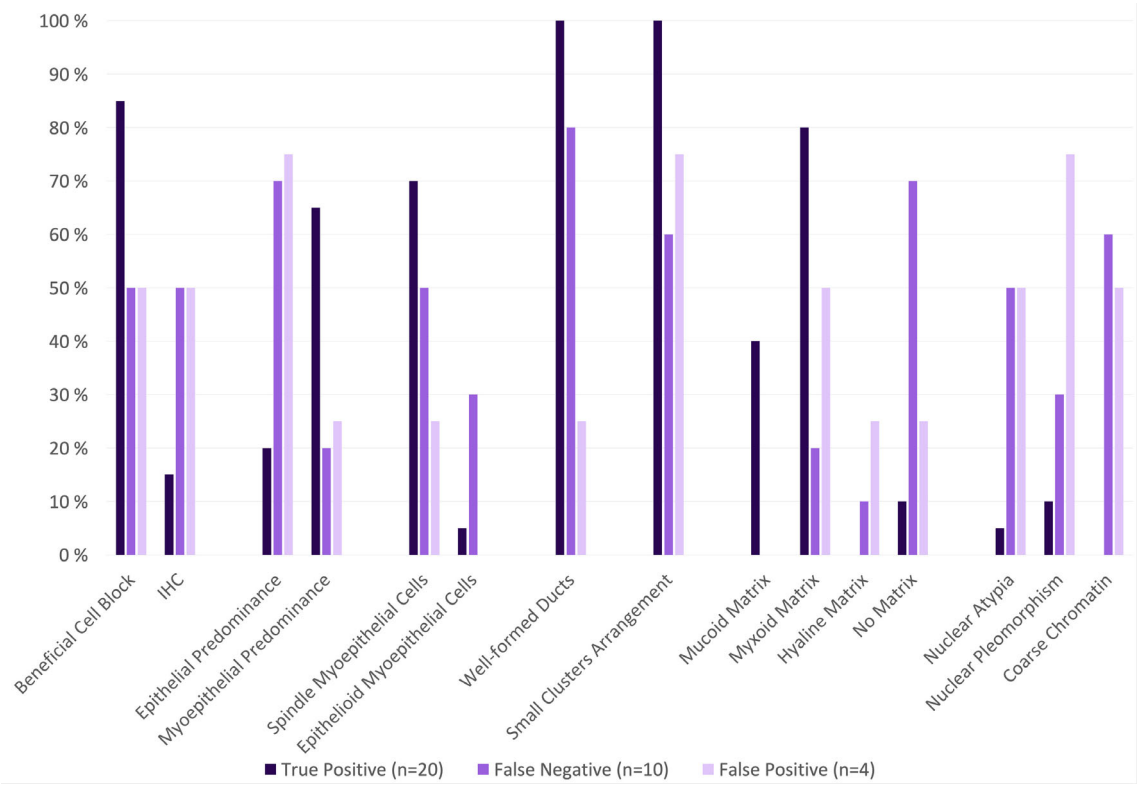

FIGURE 2 Selected cytological features in concordant and discordant cases. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Selected cytological features in concordant and discordant cases.

					False-positive		
Feature	True-positive ($n = 20$)	False-negative all ($n = 10$)	False-negative suspicious for malignancy ($n = 6$)	False-negative SUMP ($n = 4$)	Epithelial–myoepithelial ca ($n = 2$)	Mucoepidermoid ca, low grade ($n = 1$)	Carcinoma ex pleomorphic adenoma ($n = 1$)
Myoepithelial predominance	++	+/−	+	+	−	−	++
Ductal cells	++	++	++	++	−	−	++
Myxoid matrix	++	+/−	+/−	+	+	−	++
Nuclear atypia	+/−	++	++	+	++	++	−

Note: Percent of the cases: ++, common (>50%); +, rare (25%–50%); +/−, occasional (<25%); −, absent (0%).
Abbreviation: SUMP, Neoplasm of Uncertain Malignant Potential.

TABLE 5 Cytomorphological features in discordant cases.

	p-value			
Feature	False-negative all ($n = 10$)	False-negative SUMP ($n = 4$)	False-negative suspicious for malignancy ($n = 6$)	False-positive ($n = 4$)
Cellularity	0.002	0.002	0.030	0.002
Epithelial predominance	0.007	0.027	0.030	0.027
Lack of matrix	<0.001	0.003	0.004	
Nuclear atypia	0.004		<0.001	0.013

Note: *p*-values by SPSS & Cramer's *V*.
Abbreviation: SUMP, Neoplasm of Uncertain Malignant Potential.

gene 1 (*PLAG1*) on chromosome 8q12 and *High-mobility group AT-hook 2* (*HMGA2*) on chromosome 12q14.3 with various fusion partners can be detected both by fluorescence in situ hybridization and IHC, so they can be applied in FNA samples.[32,33] HMGA2 marker could help in PA and carcinoma ex PA diagnostics.[33] PLAG1 was detected both in PA and carcinoma ex PA.[34] As a nuclear marker, PLAG1 is easy to interpret in FNA material, but its diagnostic accuracy reached only 60% in FNA. The sensitivity was 55%, the specificity was 75%, and the positive predictive value was 88% in this study.[35] As also basal cell adenomas expressed PLAG1, the marker cannot be used as a single diagnostic marker of PA.[35] Elegant explanation for PLAG1 expression heterogeneity in PA was offered by Matsuyama et al.[36] Tumor cells with myoepithelial differentiation expressed PLAG1, but not those with lipomatous or squamous differentiation.[36] In a recent study ancillary tests improved diagnosis in 100% of benign neoplasm cases and in 98.3% of malignant cases.[18] In the author's experience IHC was confirmatory in schwannoma, PA with myoepithelial cell predominance or ruling out both primary and secondary carcinomas.[18] In our series there were less cases where IHC was performed mainly due to long analysis period and less IHC used and being available in earlier years, nevertheless IHC was in our experience mainly supportive. IHC was performed only in 15% of true-positive cases, but in 50% of false-negative cases and 25% of false-positive cases showing limited role of immunohistochemistry in straightforward cases with typical morphology. The MSRSGC reclassification and use of SUMP and Suspicious for Malignancy categories would clearly improve the clinical management of false negative cases. Certain proportion of PA has been and will be diagnosed as SUMP[19,28] due to various cytomorphological features.

5 | CONCLUSION

Cytomorphologically, cell type predominance was more often myoepithelial in true-positive cases (65%) and epithelial both in false-negative cases (70%) and in false-positive cases (75%). Well-formed ducts were present in cytology in all true-positive cases. Only 10% of true positive cases did not show matrix in cytology. Nuclear changes were common in false-negative cases (80%) and false positive cases (75%). The benefit of CBs was more evident in true-positive cases. Overall, the present study showed diagnostic accuracy of 96.5% for FNA in PA diagnostics. Sensitivity, specificity, positive predictive value and negative predictive value were 94.4%, 98.2%, 97.7%, and 95.6%, respectively. Based on the present analysis, PA diagnostics can be improved in pre-analytical stage by proper sampling, improved by rapid on-site evaluation.[37] Specimen would benefit from both Papanicolaou and Giemsa-based staining, CBs and ancillary technique application. Last, but not least the cytomorphological knowledge, experience and skills to avoid pitfalls is crucial to avoid misinterpretation.

AUTHOR CONTRIBUTIONS

Erkka Tommola: Data curation; formal analysis; investigation; project administration; visualization; validation; writing – original draft, editing. **Satu Tommola**: Conceptualization; data curation; investigation; supervision. **Ivana Kholová**: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; visualization; supervision; validation; writing – original draft, editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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