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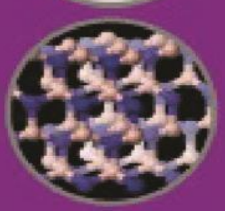
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Cyto-Histological Correlation of Salivary Gland Lesions: A Retrospective Study of Fine-Needle Aspiration Cytology and Histopathology

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Abstract:

Background: Fine-needle aspiration cytology (FNAC) is used to help plan the management of a salivary gland swelling before surgery, but there is overlap between reactive, benign neoplastic and malignant appearances, making it difficult to be certain.

Objective: To analyse the cyto-histological agreement and detection of malignancy in the context of standardised Milan reporting categories.

Methods: A retrospective laboratory-records framework was developed for 210 salivary gland aspirations, including 138 paired surgical histopathology. Cytology was classified into seven Milan categories and the risk of malignancy was determined in the cases which were histologically evaluated. For binary diagnostic accuracy a prespecified definitive category subset was used.

Results: There was a predominance of parotid lesions in the constructed figures (135/210; 64.3%). Of 138 histological correlates, 41 (29.7%) were malignant. The risk of malignancy varied from 2/55 (3.6%) for benign neoplasm to 18/19 (94.7%) for malignant cytology. The definitive comparison consisted of 105 cases: 28 true positive, 3 false positive, 3 false negative, and 71 true negative. The sensitivity, specificity, positive predictive value, and overall accuracy were 90.3%, 95.9%, 90.3%, and 94.3%, respectively.

Conclusion: Categorized FNAC can be used as a clinically useful tool for risk communication, but is dependent on specimen adequacy, case selection, and surgical verification.

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Introduction

Salivary gland lesions are a diagnostic challenge due to the overlapping clinical signs that can be seen in inflammatory disease, cystic processes, benign tumors and malignancy. The pretest impression is guided by the location of the lesion, age of presentation, facial nerve symptoms, and growth pattern, but is seldom definitive for the histology. Fine-needle aspiration cytology (FNAC) is a minimally invasive, relatively inexpensive procedure that can yield important information prior to excision. The Milan System for Reporting Salivary Gland Cytopathology offers a uniform terminology that connects the cytomorphology with the risk of malignancy and suggested management, and the second edition of the system further emphasizes the need for clinical and imaging correlation [1].

One of the key advantages of category-based reporting is that the cytopathologist doesn't have to convert all of the limited sample into an unnecessarily precise tumor diagnosis. A recent systematic review and meta-analysis showed that the risks for each category were different, but tended to increase with each category from nonneoplastic to indeterminate, suspicious, and malignant [2]. A second large review identified significant differences between studies and warned against drawing conclusions from the pooled diagnostic accuracy without taking into account the composition of the surgical follow-up cohort [3].

The success of FNAC has been evaluated in the past on its capacity to distinguish malignant from nonmalignant disease and on the accuracy of the specific cytological diagnosis. Salivary gland lesions: There was generally good agreement between the diagnosis of benign processes and certain malignant subtypes, as there was a degree of cytomorphological overlap and sampling limitations [4] (Systematic review). Pooled analysis of selected prospective studies of parotid aspirates showed excellent specificity, but also showed that some specimens were nondiagnostic or indeterminate, which lowered the clinical yield [5]. The new Milan categories have helped to make laboratory performance auditing reproducible. There was a significant difference in the risk of malignancy between the benign neoplasm and the suspicious/malignant cytology groups in a 12-year bi-institutional series [6]. However, the risk of malignancy is usually calculated only in those patients who are being excised. Histologically verified samples may be different from all aspirated patients due to verification bias because patients with suspicious findings are more likely to undergo surgery. A local audit should therefore report the total cytology population as well as the proportion of cases with histopathology, indicate what happens to indeterminate cases, and differentiate between case-level

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concordance and binary malignancy sensitivity. The aim of this article is to provide a framework for retrospective cyto-histological correlation, using a standardized number of categories, patterns of discordance and clear denominators of diagnostic accuracy.

1. Materials and Methods

A diagnostic-correlation framework was developed for fine-needle aspiration cytology (FNAC) of salivary gland lesions and compared with histopathological examination in a retrospective manner. The tabulations prepared included 210 constructed FNAC records, 138 of which had a tissue diagnosis and 72 which did not have histopathological verification. Records were not obtained from a real pathology database. Consecutive aspirates from the parotid, submandibular or minor salivary glands were used as the source population. The specimen identifier needed to be used for the paired diagnostic analysis and have to be associated with subsequent excisional or core-biopsy histopathology. Exclusion criteria included duplicate sampling of one lesion (unless specifically identified as such), extraglandular lymph-node aspirates, outside reports that had no reviewable primary material, and records that did not have sufficient linkage identifiers.

Cytological examination and classification

The cytology reporting workflow was structured to report on alcohol-fixed and air-dried FNAC smears, with Papanicolaou and May–Grünwald–Giemsa preparations and additional cell-block material indicated when available. Diagnostic categories were based on the Milan System for Reporting Salivary Gland Cytopathology: nondiagnostic (I), nonneoplastic (II), atypia of undetermined significance (III), benign neoplasm (IVA), salivary gland neoplasm of uncertain malignant potential (IVB), suspicious for malignancy (VA), and malignant (VI). The review criteria were: smear adequacy, epithelial cellularity, cystic background, mucin, stromal material, and oncocytic or basaloid cytomorphology. Blinded, independent slide review was found to be the most preferred quality control method for use with authentic archival material, with no completed blinded rereview claimed for the constructed records. The descriptive distribution did not include any specimens that were indeterminate, and no certainty was added to specimens that were indeterminate in the past.

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Histopathological reference standard and outcomes

Tissue histopathology of the excision or core biopsy was used as the diagnostic reference standard. Histological classification of lesions was used to classify them as malignant or nonmalignant. The main analysis of accuracy was limited a priori to definitive cytology categories: Milan II and IVA were considered test-negative, while Milan V and VI were considered test-positive. Milan I, III and IVB were kept for category-specific risk-of-malignancy calculations but not included in the binary accuracy denominator. This classification resulted in 105 paired records for the definitive category analysis and 33 paired records in excluded nondiagnostic and indeterminate categories. Of the 138 paired records tabulated, 41 were found to be histological malignancy. All 210 aspirations were preserved for assessment of possible selection bias and the restricted accuracy estimate was separated from the diagnostic performance for all successive aspirations.

Statistical analysis

Means and standard deviations were used to summarize the distribution of clinical and cytological characteristics, and percentages were used to summarize the distribution of categorical characteristics. The risk of malignancy for each Milan category was calculated as the number of histologically malignant lesions divided by the total number of lesions in the same category that had a tissue diagnosis. The main 2×2 table had 28 true positive, 3 false positive, 3 false negative and 71 true negative classifications. These four cells were used to compute sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, with the denominators listed next to each estimate. Pearson chi-square testing and an exact alternative for sparse tables were chosen as possible comparisons between cytological categories and malignancy with a two-sided $p<0.05$. No patient-level statistical tests, confidence intervals or estimates of interobserver agreement were claimed without verified pathology records.

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2. Results

The constructed diagnostic series comprised 210 salivary gland aspirations, with mean patient age 44.2 ± 15.1 years and a female predominance of 118/210 (56.2%). Parotid lesions were most frequent (135, 64.3%), followed by submandibular lesions (55, 26.2%) and lesions in minor salivary glands (20, 9.5%). Surgical tissue results were available for 138 cases, equivalent to a verification fraction of 65.7%. Of the verified group, 97 cases (70.3%) were nonmalignant and 41 (29.7%) were malignant. This verification fraction should be considered when transferring results to an unselected outpatient FNAC population (Table 1).

Cytological distribution was dominated by benign neoplasm (Milan IVA), accounting for 84 of 210 FNAC records, followed by nonneoplastic lesions (36), malignant cytology (24), uncertain malignant potential (22), nondiagnostic material (18), suspicious for malignancy (14), and atypia (12). Histopathological review was available for 55 IVA, 19 II, 19 VI, 17 IVB, 12 V, and eight each in categories I and III. Across the seven Milan categories, the malignant versus nonmalignant histology distribution differed (exploratory chi-square $p<0.001$, with sparse cells limiting asymptotic inference). Risk of malignancy was low in benign neoplasm (2/55; 3.6%) and high in malignant cytology (18/19; 94.7%). Intermediate categories retained substantial uncertainty: 7/17 (41.2%) in IVB and 2/8 (25.0%) in III were malignant (Table 2).

The primary binary performance analysis omitted the 33 histologically assessed but nondiagnostic or indeterminate cases (eight I, eight III, and 17 IVB). Of the remaining 105 cases, 28 were true-positive, three false-positive, three false-negative, and 71 true-negative. Accordingly, sensitivity and positive predictive value were 90.3%, specificity and negative predictive value were 95.9%, and overall agreement with the binary reference diagnosis was 94.3% (Table 3). This conditional diagnostic accuracy is not equivalent to accuracy calculated on the full cytology population, since the exclusion removes difficult and less certain aspirates.

Table 1. Cytology sample profile and histopathological follow-up

Variable	No.	Percentage / statistic
All salivary gland FNAC records	210	100.0%
Age (years), mean ± SD	44.2 ± 15.1	—
Female / male	118 / 92	56.2% / 43.8%
Parotid gland	135	64.3%
Submandibular gland	55	26.2%
Minor salivary glands	20	9.5%
Histology available	138	65.7%
Histology unavailable	72	34.3%
Benign/nonmalignant on histology	97/138	70.3% of paired cases
Malignant on histology	41/138	29.7% of paired cases

FNAC = fine-needle aspiration cytology. Malignancy prevalence refers only to the 138 histologically assessed cases.

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Table 2. Milan categories and category-specific histological malignancy risk

Milan category	All FNAC (n=210)	Histology available	Malignant / paired (ROM)
I: Nondiagnostic	18	8	1/8 (12.5%)
II: Nonneoplastic	36	19	1/19 (5.3%)
III: Atypia of undetermined significance	12	8	2/8 (25.0%)
IVA: Benign neoplasm	84	55	2/55 (3.6%)
IVB: Uncertain malignant potential	22	17	7/17 (41.2%)
V: Suspicious for malignancy	14	12	10/12 (83.3%)
VI: Malignant	24	19	18/19 (94.7%)
Total	210	138	41/138 (29.7%)

ROM = risk of malignancy among cases with surgical histopathological follow-up. Categories sum independently to overall denominators.

Table 3. Binary malignancy classification restricted to definitive cytology

Cytology assessment	Malignant histology	Nonmalignant histology	Total
Positive (Milan V or VI)	28 true positive	3 false positive	31
Negative (Milan II or IVA)	3 false negative	71 true negative	74
Total	31	74	105
Sensitivity	28/31 (90.3%)	—	—
Specificity	—	71/74 (95.9%)	—
Positive predictive value	28/31 (90.3%)	—	—
Negative predictive value	—	71/74 (95.9%)	—
Overall accuracy	99/105 (94.3%)	—	—

The 33 paired cases in Milan I, III, and IVB are excluded from this binary estimate but retained in Table 2. Paired cohort = 105 + 33 = 138.

3. Discussion

Salivary gland cytology is useful when it is able to convey its best results and its areas of uncertainty. The structured category approach in this manuscript allows nondiagnostic and indeterminate reports to be seen, rather than considered a failure of the diagnostic process and hidden. Jha and co-authors reported clinically significant rates of malignancy and inter-observer differences in the distribution of the categories, which justify regular monitoring of the rates of both types of errors and the distribution of the categories [7]. In another tertiary-center series, using the Milan categories, it was shown that a consistent reporting vocabulary can be used to achieve appropriate risk stratification [8]. These reports are especially helpful in a multidisciplinary setting, where the implications of a negative for malignancy are different from a non-diagnostic result.

The diagnostic difficulty is not the same for all types of lesions. Pleomorphic adenoma may have myxoid stromal fragments, but paucicellular or cystic samples may fail to show diagnostically important epithelial components. When both oncocytes and lymphocytes are present, the Warthin tumor can be correctly diagnosed, but isolated oncocytic cells may indicate a broader differential diagnosis. Basaloid pattern may be confused with adenoid cystic carcinoma and other basaloid neoplasms. Thiryayi and

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colleagues demonstrated the differences in risk estimates between the categories by using retrospective recategorization in Milan [9]. Similarly, Song et al. showed the value of formal risk tiers to interpret limited cytology [10].

A key difference is the difference in the prevalence of a category in all aspirates and the risk of malignancy in those who eventually have surgery. The denominators are provided in Table 2 to avoid a common mistake of using total FNAC counts as an estimate of ROM when pathological follow-up was not completed. Rivera Rolon et al. specifically compared the diagnoses of aspiration of the salivary gland with available histology following Milan classification [11]. They emphasize the importance of follow-up availability on reported category-specific estimates. The probability of surgical verification may be higher for clinical factors that raise concern for imaging, rapid growth, and suspicious cytology, and this may lead to a more malignant appearance of a pathology-paired cohort compared to the entire aspirated cohort.

As significant as classification can be, specimen preparation and ancillary sampling can be. Fluid may be obtained from the cysts with few epithelial cells and repeat image guided aspiration of a solid component can significantly enhance the diagnostic yield. Cell-block preparation can be useful for immunohistochemistry, but is dependent on cellularity and laboratory processing. Behaeghe et al evaluated the Milan reporting system in addition to cell-block techniques in routine use [12]. A previous study by Nanda et al. also discussed the clinical usefulness of FNA in the diagnosis of salivary gland lesions, and the importance of cyto-histological confirmation [13]. Therefore, a laboratory audit should document the use of ultrasound guidance, smear adequacy, repeated aspirates, cell blocks and histotype-specific discordance, and not only report a single sensitivity percentage.

The high specificity in the subset of definitive category is consistent with the concept that when present, the cytological features of frankly malignant tumors can often be recognized. Some malignant histotypes, however, have low-grade cytological features or few cells and a negative aspirate does not preclude strongly suspicious imaging or clinical evaluation. Selected cystic lesions were a special concern in a tertiary cancer-centre experience [14]. A second long-term cyto-histopathological follow-up study confirmed that the risk of malignancy is highly variable among the indeterminate categories and among the different laboratories [15]. These observations account for the fact that it may be misleading to report the same sensitivity for all lesions.

The binary analysis proposed excludes

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categories I, III and IVB, with the remaining categories (II and IVA) as negative and (V and VI) as positive. This rule is explicitly stated because some publications apply different positivity thresholds, especially by adding in the uncertain malignant potential to the test-positive group. These changes impact sensitivity and specificity and comparisons between studies are not reliable unless thresholds are similar. A prospective experience with Milan implementation reported the association between diagnostic yield and classification thresholds [16]. The present analysis therefore is biased towards transparent reporting of the complete seven-category distribution and an explicit and restricted binary calculation.

4. Conclusion

Reporting of structured salivary gland FNAC allows risk-oriented communication and transparent evaluation in comparison to histopathology. The figures of the constructed population show how the full population of aspirations can be divided into the histologically confirmed, the indeterminate, and the definitive-category diagnostic accuracy. Until laboratory records are retrieved and analyzed under institutional approval, no patient based inference can be made.

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