



# Two-Year Experience of the Implementation of the Milan for Reporting Salivary Gland Cytopathology at a Private Medical Laboratory

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## Abstract

This study aimed to present the 2-year experience of the implementation of the Milan System for Reporting Salivary Gland Cytopathology at Alpha Prolipsis Medical Laboratories, a private medical laboratory located in Athens, Greece. A total of 102 Fine Needle Aspirations (FNAs) performed since 2018 were included in the study. Reports were issued according to the Milan System for Reporting Salivary Gland Cytopathology. Aspirates were prepared with both conventional and liquid-based cytological methods and were evaluated by two or three Board-certified cytopathologists. Diagnostic reproducibility and accuracy were evaluated. All cases included in this study had histologic follow-up. The diagnostic accuracy of FNA for differentiating between benign and malignant disease according to MSRSGC classification was 93.3%, the specificity was 97.5% and the sensitivity was 82.2%. The positive and negative predictive values were 93.2 and 87.2%, respectively. Our results show that FNA is a valuable examination technique in the preoperative evaluation of salivary gland lesions. The integration of the 2018 Milan System for Reporting Salivary Gland Cytopathology is effective, with an overall accuracy around 95%.

**Keywords** Salivary glands · Fine needle aspiration · The Milan system for reporting salivary gland cytopathology · MSRSGC · Risk of malignancy · Reproducibility · Liquid-based cytology

## Introduction

Despite the proven efficacy and acceptance of fine-needle aspiration (FNA) in the pre-operative evaluation of tumors, the cytopathology of salivary glands presents major challenges because of the large heterogeneity of the reported neoplasms, as it is reflected in World Health Organization 2017 Classifications [1]. Despite the fear of facial nerve injury or tumor seeding, FNA for salivary gland tumors is still the favored approach with good sensitivity (83–92%) and specificity (93–100%), since clinical examination alone usually cannot distinguish between a salivary gland tumor, an inflammatory process, or an enlarged lymph node, and the available imaging techniques, such as three-dimensional computerized tomography, magnetic resonance imaging and ultrasonography cannot reveal the exact nature of salivary gland tumors [2, 3].

FNA is a non-traumatic and safe diagnostic procedure which is well tolerated by the patient, can be instantly repeated and may be used for the execution of several pre-operative ancillary studies [2, 3]. Pre-operative FNA can be used in order to make the initial diagnosis, differentiate benign from malignant tumors, confirm a suspected malignancy, and verify recurrence of a previously treated neoplasm [2, 3]. The accuracy of FNA for salivary gland tumors is comparable to that of frozen section diagnosis, which remains the diagnostic mainstay for guiding the surgeon's operative procedure but gives no assistance in pre-operative planning, as FNA does [2, 3].

Until 2018, in many FNA reports, for heterogeneous tumors, with overlapping cytological features, it was not possible to accurately classify them into distinct diagnostic categories, partly because of factors such as the lack of a widely accepted standardized reporting format and the use of multiple, often overlapping, cytological terms in descriptive reports lacking a definite diagnosis [4, 5].

A worldwide accepted reporting system providing proper risk-stratification (in which the likelihood of malignancy is provided for each diagnostic category) is needed in order to provide definite indications concerning patient management.

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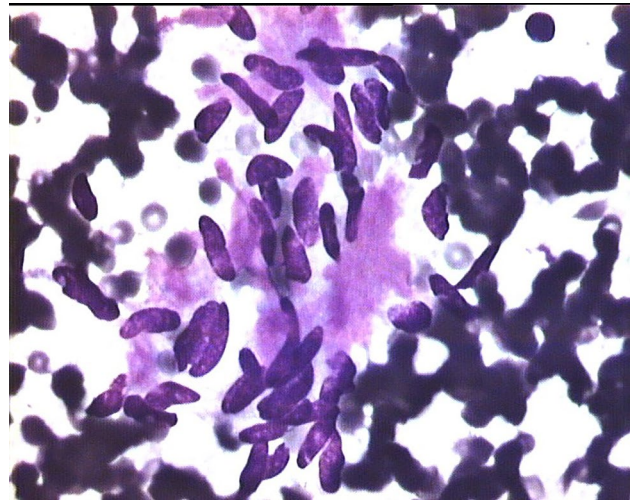
The proposed reporting system should be easy in everyday practice and guarantee good intra- and inter-observer reproducibility for each diagnostic category [4–6].

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was introduced in 2018 by the American Society of Cytology and the International Academy of Cytology in order to standardize terminology used in reporting salivary gland cytology [4–6]. This Milan System for Reporting Salivary Gland Cytopathology is based on the experience of experts in the field of cytopathology and on evidence from the literature. The seven categories used, namely non-diagnostic, non-neoplastic, atypia of undetermined significance, benign neoplasm, salivary gland neoplasm of uncertain malignant potential, suspicious for malignancy and malignant, are supplemented by a list of diagnostic criteria. Besides classification, a precise identification of the neoplasm should also be indicated, as well as a clear distinction between low-grade and high-grade malignancies [4–6].

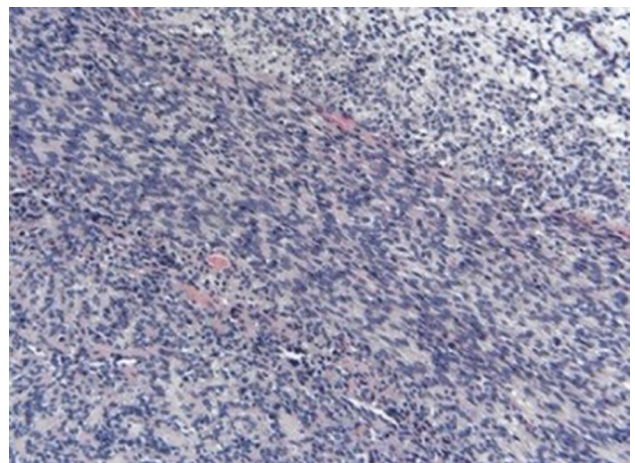
The objective of this study was to present the 2-year experience of the implementation of the Milan System for Reporting Salivary Gland Cytopathology at Alpha Prolipsis Cytology Laboratories, a private medical laboratory located in Athens (Greece) and to present internal quality control measures that were implemented in order to increase reliability and traceability of cytological findings and reports.

## Materials and Methods

The study included patients with palpable and non-palpable salivary gland masses referred to Alpha Prolipsis Cytology Laboratories during a 2-year period. The laboratory is certified according to ISO 15189:2012 and employs three Board-certified cytopathologists with well-documented experience in salivary gland cytology. Since April 2018, Alpha Prolipsis Cytology Laboratories has reported all salivary gland Fine Needle Aspirations (FNAs) using the Milan system and followed the guidelines in the diagnostic manual “The Milan System for Reporting Salivary Gland Cytopathology” [4]. A total of 102 cases of salivary gland FNAs were examined. The patients were directly referred to Alpha Prolipsis Cytology Laboratories. All FNAs were performed under ultrasound guidance by a consultant radiologist. All aspirations (usually three or four passes per lesion) were performed under ultrasound guidance with 21-gauge needles attached to a 10-cm syringe for suction. On-site evaluation of the specimen adequacy was performed in all cases by staining by Giemsa-quick stain (Hemacolor, Merk, Darmstadt, Germany). Multidirectional withdrawal of the needle alone through the lesion usually yielded sufficient material to establish a diagnosis. In the case of non-diagnostic



**Fig. 1** Spindle cell pleomorphic adenoma (MGG stain, magnification  $\times 400$ ) initially diagnosed as salivary gland neoplasm of uncertain malignant potential

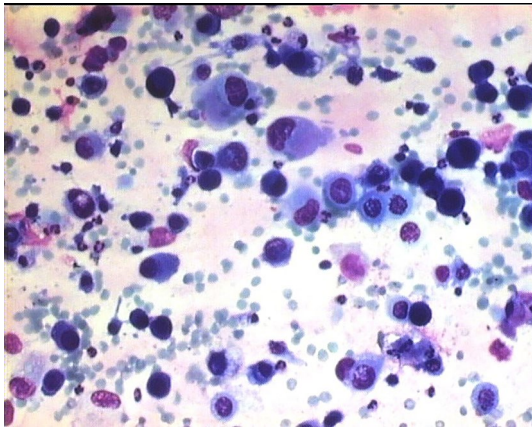


**Fig. 2** Spindle cell pleomorphic adenoma (hematoxylin–eosin, magnification  $\times 100$ )

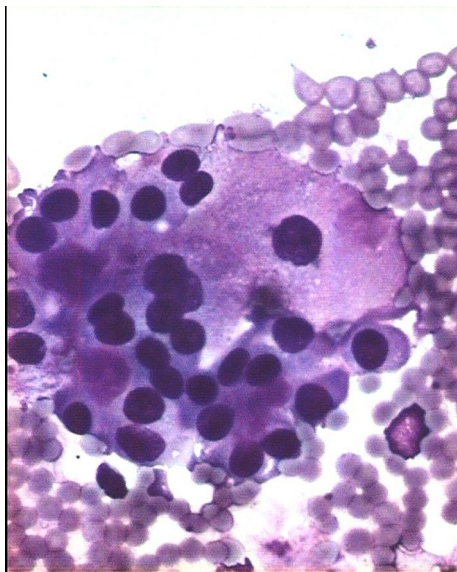
sampling, immediate repeat FNA was mandated, at times using the syringe for suction (Figs. 1, 2, 3, 4).

Smears were made with both conventional and liquid-based cytological methods and were stained with both May-Grunwald-Giemsa and Papanicolaou stains. All slides were diagnosed simultaneously by two or three Board-certified cytopathologists. Immunocytochemical reactions with a panel of monoclonal antibodies were available against epithelial, lymphoid or melanoma markers. All antibodies were from Dakopatts (Glostrup, Denmark) and were used with the two-step alkaline-anti-alkaline phosphatase method.

All cytopathologists used the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) terminology and



**Fig. 3** Mucoepidermoid carcinoma (PAP stain, magnification  $\times 400$ ) initially diagnosed as salivary gland neoplasm of uncertain malignant potential



**Fig. 4** Adenoid cystic carcinoma (MGG stain, magnification  $\times 400$ ) initially diagnosed as pleomorphic adenoma

adhered to its diagnostic criteria. Whenever diagnostically challenging cases were encountered, the final diagnosis was made after teleconsultation with an expert colleague with well-known experience in the field.

Histological reports were methodically collected, reviewed and compared with initial cytological diagnoses for all 102 cases. Malignancy rates for each MSRSGC category were calculated. The sensitivity and specificity of cytology for a histological diagnosis of malignancy was assessed. Statistical processing was performed with the software package IBM SPSS Statistics v.19 (IBM Corporation, Armonk, NY, USA).

## Results

A total of 102 patients underwent salivary gland Fine Needle Aspirations (FNAs) during the study period; 58 (56.9%) male and 44 (43.1%) female patients, with a median age of 52.1 years (range = 19–80 years) and a median size of aspirated masses of 1.8 cm were included in our study. The incidence of each Milan System category for reporting salivary gland cytopathology is summarized in Table 1.

All 102 patients had histopathology available for review, resulting in non-neoplastic lesions accounting for 12 out of 102 cases (11.8%), whereas 90 lesions (88.2%) were neoplastic. Of the 90 neoplastic lesions, 66 (70%) were benign, and 24 (30%) were malignant. Correlation between cytological and histological diagnoses are summarized in Table 2.

There was one non-diagnostic case of pleomorphic adenoma. The cytological diagnosis was true-positive in 11 out of 12 cases (91.7%) and true-negative in 79 out of 90 cases (87.8%). There were 10 false-negative results (10%) and one false-positive result (1.1%). The single false-positive case, which was diagnosed as carcinoma on cytology, turned out to be atypical pleomorphic adenoma.

Of the lesions included in non-neoplastic cases (category II) two were abscesses, one was a cyst, seven were sialadenitis, one carcinoma ex pleomorphic adenoma, one adenoid cystic carcinoma, one mucoepidermoid carcinoma, two Warthin's tumors and three pleomorphic

**Table 1** Salivary gland cytopathology according to the Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) with the risk of neoplasm and malignancy

Diagnostic category	Incidence	Risk of neoplasm	Overall risk of malignancy
I: non-diagnostic	3/102	2/3 (66.6%)	1/3 (33.3%)
II: non-neoplastic	18/102	8/18 (44.4%)	3/18 (16.7%)
III: atypia of undetermined significance	1/102	1/1 (100%)	1/1 (100%)
IVa: neoplasm: benign	65/102	65/65 (100%)	5/65 (7.7%)
IVb: neoplasm: salivary gland neoplasm of uncertain malignant potential	2/102	2/2 (100%)	1/2 (50%)
V: suspicious for malignancy	1/102	1/1 (100%)	1/1 (100%)
VI: malignant	12/102	11/12 (91.7%)	11/12 (91.7%)

**Table 2** Diagnostic correlation between cytological and histological reports

	Milan system category (n)						Total	
	I	II	III	IVa	IVb	V		VI
Pleomorphic adenoma	1	3		37	1		1	43
Monomorphic adenoma				2				2
Oxyphilic adenoma				1				1
Warthin’s tumor		2		20				22
Abscess		2						2
Granulomatous sialadenitis	1	7						8
Retention cyst		1						1
Mucoepidermoid carcinoma		1	1	1	1		5	9
Adenoid cystic carcinoma	1	1		1			3	6
Carcinoma ex pleomorphic adenoma		1		3		1	2	7
Metastatic carcinoma							1	1
<b>Total</b>	<b>3</b>	<b>18</b>	<b>1</b>	<b>65</b>	<b>2</b>	<b>1</b>	<b>12</b>	<b>102</b>

adenomas. The cytological identification of high-grade features raising suspicion of malignancy was not feasible in the material aspirated from the above mentioned lesions.

There was one case reported as a category III lesion (atypia of undetermined significance). On histopathology, it turned out to be low-grade mucoepidermoid carcinoma. The cytological identification of high-grade features raising suspicion of malignancy was not feasible in the material aspirated from this tumor.

There were 65 cases categorized as benign neoplasms (IVa) and 2 cases categorized as neoplasms of uncertain malignant potential (IVb). Six of them (5 of the IVa and 1 from the IVb category) were found to be malignant on histopathology. These were two cases of mucoepidermoid carcinoma, three carcinomas ex pleomorphic adenoma and one adenoid cystic carcinoma. On cytology an attempt was made to identify the presence or absence of high-grade features in all examined cases. The cytological identification of high-grade features raising suspicion of malignancy was not feasible in the material aspirated from the majority of these lesions. In only 2 cases there were minor cytological findings suggesting a probable malignant potential of the examined neoplasm (cells in clusters and singly scattered, round to oval cells, with a pleomorphic vesicular nucleus, prominent nucleoli, and scanty cytoplasm) which correlated well with the final histological diagnosis.

In our study, one category V (Suspicious for malignancy) case was reported as a neoplasm on cytology with the possibilities of atypical pleomorphic adenoma versus carcinoma ex pleomorphic adenoma. Malignancy was histologically confirmed.

Among 12 malignant neoplasms (Category VI), 11 were confirmed, while there was one false-positive diagnosis of carcinoma on cytology and atypical pleomorphic adenoma on histology. One metastatic thyroid follicular carcinoma

was diagnosed by immunocytochemistry (positivity to Thyroid Transcription Factor 1).

A statistical analysis of the results was performed. In this study, the diagnostic accuracy of FNA for differentiating between benign and malignant disease was 93.3%, the specificity was 97.5% and the sensitivity was 82.2%. The positive and negative predictive values were 93.2 and 87.2%, respectively. The risk of malignancy (the percentage of cases that were proved histologically malignant) was calculated according to MSRSGC diagnostic categories (Table 2). The criteria used to define true positive and true negative cases were referring to the distinction benign versus malignant lesions. True positives were all cases in which initial cytological diagnosis of malignancy (categories 5 and 6) was histologically confirmed. True negatives were all cases in which initial cytological diagnosis of non-malignancy (including both neoplastic and non-benign lesions of categories II, III and IV) was histologically confirmed.

### Discussion

The management of salivary lesions varies based on the underlying histology [7, 8]. Preoperative diagnosis can guide the decision for surgery and the extent of resection. Surgery is often not indicated for non-neoplastic salivary gland lesions, for metastatic cancer and for malignant lymphoproliferative disease. Fine Needle Aspiration (FNA) is a well-recognized safe, accurate, and cost-effective method for immediate evaluation of salivary gland lesions and can improve and facilitate clinical management of patients by providing valuable information concerning the nature of the lesion examined [2, 8, 9].

The Milan System for Reporting Salivary Gland Cytology (MSRSGC), which was adopted in 2018, is an excellent tool for the diagnosis and management of salivary gland

lesions [4, 5]. Its main objective is to improve communication between clinicians and cytopathologists and facilitate overall patient management. It is an evidence-based system which provides clinical management strategies for each category [5, 6]. MSRSGC has classified salivary gland cytological diagnoses into the following categories: non-diagnostic, non-neoplastic, atypia of undetermined significance, benign neoplasm/salivary gland neoplasm of uncertain malignant potential, suspicious for malignancy and malignant, with an estimated risk of malignancy of 25, 10, 20, 5, 35, 60, and 90% for each category [4–6]. Our study also categorized salivary gland FNA into six categories according to MSRSGC, and the risk of malignancy reported was 33%, 16.7, 100, 7.7, 50, 100, and 91.7%, respectively for each category. These results are comparable to that provided in MSRSGC, except for category III, in which there was only one case available for statistical analysis.

Our study's findings, regarding FNA accuracy and sensitivity are also consistent with older studies findings. The sensitivity of FNA, as mentioned in different previous studies, ranges from 54 to 98%, with high specificity values of 88–99% for separating benign lesions from malignant lesions [3, 10–12]. In the current study, utilizing MSRSGC for diagnosing malignant lesions by FNA, the diagnostic accuracy of FNA for differentiating between benign and malignant disease was 93.3%, whilst the specificity 97.5% and the sensitivity 82.2%. The positive and negative predictive values were 93.2 and 87.2%, respectively.

Category I (Non-diagnostic) cases are non-diagnostic salivary gland aspirates providing insufficient diagnostic material [13]. Factors such as the presence of marked fibrosis, marked cystic change and acellular aspirates may lead to a false-negative diagnosis. In salivary gland cystic lesions such as retention cyst, mucocele, lymphoepithelial cyst, Warthin's tumor, mucoepidermoid carcinoma, acinic cell carcinoma, cystic pleomorphic adenoma or cystadenoma, the cellularity of the smear is often low and malignant cells can be missed [7, 12, 13]. Post-evacuation FNA with multiple passes from different planes can substantially reduce sampling errors.<sup>2</sup> The accuracy of FNA is also affected by the experience of the person assigned to performing the procedure [2, 3]. Failure to obtain a representative sample caused by needle positioning outside the target tissue, central necrosis, or hemorrhage or cystic change in the tumor may lead to a false-negative diagnosis [2, 3, 11, 12]. Non-diagnostic smears can be reduced if a cytopathologist is available to examine the smear immediately and to repeat the procedure in the case of there being inadequate material [14]. This is the procedure followed in our study and explains why we had only three final cytological diagnoses of inadequate material and one cytologically suspicious lesion.

Category II (Non-neoplastic) cases are non-neoplastic salivary gland aspirates [13]. A benign parotid mass can

be due to an inflammation, a cyst, or an intraparotid lymph node [2, 3]. Main reasons of false negative results was scant cellularity of smears, presence of hemorrhage and inflammation in the background, obscuring cytological findings [2, 3, 12]. Factors such as the presence of cystic change, histiocytes, and foreign body granulomatous reaction may lead to a false-negative diagnosis [2, 3]. When inflammatory material is aspirated, it is always feasible to administer antibiotics and repeat FNA 2–3 weeks later [2, 14].

Category III (Atypia of undetermined significance) cases are those in which a neoplastic lesion cannot be completely ruled out [13]. The presence of cellular atypia may lead to categorization into atypia of undetermined significance on FNA evaluation [13]. In our opinion, the use of this diagnostic category should be minimized and applied to cases where cytology is truly unable to rule out malignancy.

Category IVa (Neoplasm: Benign) cases are those in which a benign neoplastic lesion is identified and IVb (Neoplasm: Salivary gland neoplasm of uncertain malignant potential) cases are those in which cytological findings cannot reliably distinguish between a benign and malignant neoplasm [13]. FNA can reliably distinguish benign and malignant neoplasms with high specificity [3, 14, 15]. However, differentiation between a benign neoplasm and low-grade malignant neoplasm may be very difficult in some cases [3, 14, 16]. Although benign pleomorphic adenoma can be easily recognized, there are cases where the aspirated material is full of mucin, lacking the characteristic tumor cells [2, 14]. A detailed examination may reveal a few characteristic cells, yet a definite diagnosis in these cases is rather difficult [2]. Atypia of the epithelial cells is not uncommon in pleomorphic adenomas and is not an indication of malignancy [2, 16]. Only the coexistence of poorly differentiated cells will guide diagnosis towards a malignant tumor [2, 17]. Typical Warthin's tumor, by virtue of its biphasic cellularity, may be identified accurately [2, 18]. However, in the presence of extensive squamous metaplasia, the differential diagnosis from squamous cell or mucoepidermoid carcinoma may be difficult [2, 19]. The aspiration of numerous masses of oncocyctic cells can lead to the misinterpretation of Warthin's tumor as oxyphilic adenoma [2].

Category V (Suspicious for malignancy) cases are those in which cytological findings are suggestive of malignancy [13]. This diagnostic category has been used by cytopathologists for a long-time and is well-known to clinicians. Our laboratory's policy was to minimize its use only in cases where cytology was really unable to rule out malignancy. The presence of three experienced cytopathologists as well as the availability of teleconsultation were contributing factors to the small number of cases that were classified as category V in our study.

Category VI (Malignant) cases are those in which cytological findings are diagnostic for malignancy [13]. The most

difficult type of malignant neoplasm to diagnose cytologically is mucoepidermoid carcinoma, followed by acinic cell carcinoma [2, 3, 19]. The distinction between a primary and a metastatic tumor is supported, to a large extent, by immunocytochemistry and the patient's history [2, 20]. Prior studies have reported a wide range of results on the accuracy of FNA for detecting malignancy, with sensitivities ranging from 33 to 100% and specificities ranging from 67 to 100% [16, 19, 20]

Many factors played a significant role in the satisfactory results of our study. The use of liquid-based cytology techniques was proven valuable, especially in cases where special immunocytochemical stains were applied for diagnostic purposes. The use of image-guided methods, the high experience of the radiologists involved in the sampling procedure, as well the adequate training of laboratory personnel on slide preparation and staining are factors that resulted in excellent diagnostic results.

Our laboratory has been accredited since 2012 according to EN ISO 15189:2012. According to this international quality standard, as long as the number of mistakes committed during specimen collection, preparation, and diagnostic interpretation diminishes, all monitored quality assessors continue to improve, and vice versa [21]. The laboratory is continuously monitoring factors such as interobserver and intraobserver agreement, which play crucial role in diagnostic reproducibility. The use of static tele-cytology applications for teleconsultation purposes was shown to be an excellent alternative method for acquisition of expert opinion in diagnostically challenging cases. The most common manifestation of interobserver discrepancy is up-grading of a cytological diagnosis to a that of definitive carcinoma or down-grading of a suspicious cytological diagnosis to that for a rather benign lesion [21]. The cytopathologists of our laboratory have adequate experience in interpreting salivary gland cytology and are continuously monitored by means of internal quality control measures in order to improve and enhance their diagnostic capacities by all available means, such as participation in educational activities, daily discussions on scientific topics concerning the application of MSRSGC in everyday laboratory practice.

Our study demonstrated a high sensitivity and specificity of the MSRSGC, even during the first years of its implementation. Proper clinical management according to FNA findings can still be improved in order to avoid unnecessary surgeries and to ensure that MSRSGC is applied by both clinicians and cytopathologists as an indispensable interactive collaboration tool, diminishing clinical risks and ensuring patients' best interest.

Despite the well-known heterogeneity and morphological overlap between different salivary gland lesions, MSRSGC can improve cooperation among cytopathologists and clinicians because besides risk stratification of malignancy, it

can reduce false-negative and false-positive diagnoses by placing salivary gland FNA into well-defined diagnostic categories. This may be extremely beneficial, especially in cases with overlapping cytological features, where the use of the risk-stratification classification can lead the treating clinician towards the most appropriate management strategy.

**Author Contributions** All authors conceived of the presented idea. SA performed the computations and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

## Compliance with Ethical Standards

**Conflicts of Interest** The authors have no conflict of interest to declare.

**Ethics Approval** The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments and was approved by ALPHA PROLIPSIS Cytology laboratories research ethics committee.

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