RESEARCH ARTICLE

Diagnostic accuracy of fine needle aspiration cytology in parotid gland lesions

Hajiioannou J[1], Gkrinia E[1], Brotis GA[2], Saratziotis A[1], Nepka C[3], Korais C[1], Skoulakis C[1]

[1]Department of Head and Neck Surgery
[2]Department of Neurosurgery
[3]Histopathology Department
University Hospital of Larissa, Larissa, Greece

Abstract

Background: This study aimed to estimate the fine needle aspiration cytology's (FNAC) diagnostic accuracy in differentiating neoplastic from inflammatory lesions (Q1) and malignant from their benign counterparts (Q2).

Methods: We present a retrospective case series covering a single University Hospital and six attending head and neck surgeons over eight years (January 2011 to July 2017). We concentrated on adults with clinically suspected parotid gland lesions. We offered all patients FNAC biopsy preoperatively, and the final diagnosis was established based on the findings of the final histology. The FNAC and histology results were cross-tabulated in a 2 x 2 contingency table, from which we calculated the diagnostic accuracy, sensitivity, specificity, and positive and negative predictive values.

Results: From 212 consecutive patients reviewed, and after excluding thirteen cases (8 %) of valid but non-diagnostic FNAC, 161 cases (50 females and 111 males) fulfilled set eligibility criteria. The most common diagnosis was Warthin tumors (53 patients, 34 %), followed by pleomorphic adenomas (52 patients, 33.5 %). The sensitivity and specificity of FNAC in differentiating neoplastic from non-neoplastic lesions and in segregating malignant from benign conditions were estimated to be as high as 50 % and 97 %, and 98 % and 93 %, respectively.

Conclusion: FNAC is moderately effective in differentiating non-neoplastic from neoplastic disease and highly accurate in selecting malignant lesions from benign ones. Although the lack of FNAC sensitivity can occasionally be problematic, it still comprises a valuable tool in salivary gland surgery. HIPPOKRATIA 2022, 26 (1):25-31.

Keywords: Fine needle aspiration cytology, FNAC, diagnostic accuracy, sensitivity, specificity, parotid gland, salivary gland tumors, malignant parotid tumors, salivary gland surgery

Corresponding author: Jiannis Hajiioannou, MD, PhD, MSc, Professor of Otorhinolaryngology - Head and Neck Surgery, ENT Department, Faculty of Medicine, School of Health Sciences, University of Thessaly, University Hospital of Larissa, Biopolis, 41110, Larisa, Greece, tel: +302413502740, +306945792028, e-mail: jhajiioannou@gmail.com

Introduction

Tumors of the salivary glands account for around 3-6 % of head and neck tumors, with their incidence rate reported at 0.4-13.5 cases per 100,000 per year[1]. Up to 85 % of salivary gland neoplasms originate in the parotid gland, whereas most of them, approximately 75 %, are benign[2]. Their management may vary depending on the histopathological type, including surgical and/or conservative treatment[3]. Hence, accurate early diagnosis of potential neoplastic lesions, especially the differentiation between benign and malignant tumors, is of great importance[4].

Since both benign and malignant salivary gland neoplasms arise mainly as painless masses, their clinical evaluation must be supplemented by diagnostic imaging[2]. Many imaging modalities have been utilized for the diagnosis of salivary masses, including ultrasound (U/S), computed tomography (CT), and magnetic resonance imaging (MRI)[5]. However, obtaining an accurate preoperative diagnosis is frequently challenging, while histopathologically examining the excised tumor constitutes the gold standard for the definite diagnosis[3]. Since not every salivary gland lesion requires surgical treatment, an open excisional biopsy would be a theoretical option. However, it is not advised since it is associated with many significant complications, including damage to the facial nerve, tumor spillage, scarring, great auricular nerve paresthesia, and fistula formation[4].

Fine needle aspiration cytology (FNAC) is widely employed in preoperatively assessing salivary gland lesions[6]. It is a minimally invasive and easy-to-perform technique due to the anatomical accessibility of salivary glands. Evaluation of the smear is immediate, and the procedure is repeatable to obtain enough tissue for diagnosis or special investigations[7,8]. It is a widely accepted method since it is inexpensive, rapid, safe, relatively painless, and well tolerated in general[9]. Additionally, it has been reported that FNAC can obviate surgical treatment necessity in as many as one-third of patients with non-neoplastic lesions[10-12]. However, even if FNAC is considered a reliable technique to differentiate benign from malignant tumors, the overlap of the cytomorphologic features detected between benign and low-grade malignancies diminishes its efficiency in specifying the surgical management that is required[8].

Despite the numerous investigating studies regarding its accuracy, the value of FNAC in diagnosing parotid gland lesions remains controversial, mainly due to its low sensitivity, given that sensitivity and specificity have been reported at 33-100 % and 67-100 %, respectively[8,13]. This study aimed to estimate FNAC's diagnostic accuracy in differentiating neoplastic from inflammatory lesions (Q1) and malignant from their benign counterparts (Q2) in a tertiary University Hospital setting.

Methods

Study design

A retrospective case-series study was conducted based on the review of respective hospital records from a tertiary Head-and-Neck Surgery center. The Institutional Review Board of the University Hospital of Larissa approved the study (decision No: 14/14th, date: 09/11/2021). All participants' data were handled according to the Helsinki as well as the Health Insurance Portability and Accountability Act (HIPAA)[14,15]. For this retrospective design, no informed consent was deemed necessary, and the study extended over eight years (January 2011 to July 2017), covering a single institution and six attending head and neck surgeons. The specific period was chosen due to the patient's data availability during the study design.

Participants

All consecutive patients with benign or malignant parotid gland tumors surgically managed in our institution were evaluated as eligible for the study. Specifically, in our retrospective study, we included: i) adults (older than 18 years), ii) with clinically suspected parotid gland lesions, iii) that presented at the University Hospital of Larissa and underwent a partial or total parotidectomy, iv) during the study period (January 2011 to July 2017). In contrast, we considered ineligible patients with i) recurrent lesions of the parotid gland, ii) severe comorbidity that precluded surgical management, iii) non-diagnostic FNAC biopsy results, iv) incomplete medical data regarding preoperative and postoperative diagnosis (unavailable information that could not be retrieved either from the Otolaryngology-Head and Neck Surgery department or the Histopathology department), and v) pediatric cases. We calculated the sample size with a confidence level of 95 % and a margin of error of 5 %[16]. All patients during their hospitalization received the standards of care according to the Institutional Protocol and the National Comprehensive Cancer Network (NCCN) Guidelines[17].

FNA technique

We perform FNAC with a 23-gauge needle attached to a 20 ml syringe for all patients with clinically suspected salivary gland lesions; usually, two to four consecutive passes are performed targeting the lesion. Ultrasound guidance is utilized for lesions that are not readily palpable. Aspirated material was transferred on a clean glass slide and spread as thin and even film. Multiple smears are prepared, and materials are fixed in alcohol for hematoxylin-eosin (H&E) stain. Smears were air-dried and fixed in methanol for Giemsa staining[18]. All smears were examined and interpreted at the Department of Cytopathology of the University Hospital without knowledge of the results of the permanent histology diagnosis. Preoperative cytological findings of FNAC were classified as benign, non-diagnostic, and malignant.

Histology technique

The surgery took place at least fourteen days after the FNAC procedure. All surgical specimens excised during surgery were sequentially sent to the Pathology Department. They were reviewed by an experienced but blinded histopathologist unaware of the FNAC result, so the preoperative diagnosis does not influence the final histopathology result's accuracy. Each specimen was fixed in buffered formalin, and embedded in paraffin. A thin section cut and stained by H&E stain was used to evaluate its adequacy and architecture. The slides of all cases were examined under a light microscope to reach a definitive diagnosis. We established the final diagnosis based on the surgical specimen's histologic findings without knowing the FNAC outcome. The histopathology results were initially categorized as inflammatory (non-neoplastic) and neoplastic lesions. We defined inflammatory lesions as those with histopathologic findings of benign acinar and/or ductal epithelium, with metaplastic, reactive changes or inflammatory evidence suggesting acute (interstitial edema, limited infiltration by granulocytes, lymphocytes, and monocytes of the periductal and intracinar connective, edema of acinar and ductal epithelium) or chronic sialadenitis (acinar atrophy, lymphoid infiltration, fibrosis, ductal dilatation, and lining epithelium hyperplasia). We defined neoplastic lesions as those with cytomorphologic evidence of abnormal and uncontrolled growth of tissue[10,19]. Then, neoplastic lesions were classified as benign (non-cancerous) and malignant (cancerous) lesions based on their diagnosis, according to the WHO's histological classification of salivary gland tumors[19].

Data extraction

Two residents (GE, KC) reviewed the hospital charts of all eligible patients, and the following data were extracted: i) demographic data, including the patient's age and gender, ii) the FNAC results, and iii) the permanent histology results.

Statistical analysis

Cases without FNA results were treated as "missing results" and excluded, while those without a permanent histological diagnosis were treated as "uninterpreted results" and sequentially excluded. The non-diagnostic FNAC biopsies were treated as "valid inconclusive results" that were analyzed as an independent category. From the remaining cases, we cross-tabulated the results of FNAC and permanent histology in a 2 x 2 contingency table to calculate the true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Afterward, we calculated the diagnostic accuracy, sensitiv-

ity, specificity, positive predictive value, and negative predictive value of FNA biopsy in comparison to final histopathology for the diagnosis of parotid gland lesions, in accordance with the following diagnostic accuracy parameters: sensitivity (Sen): TP/(TP+FN), specificity (Spec): TN/(TN+FP), accuracy (Acc): (TP+TN)/total, positive predictive value (PPV): TP/(TP+FP), negative predictive value (NPV): TN/(TN+FN), positive likelihood ratio (LR+): Sen/(1-Spec), and negative likelihood ratio (NR-): (1-Sen)/Spec. Fagan's nomogram[20] estimated the post-test probabilities. The diagnostic accuracy calculations were performed separately for questions Q1 and Q2. All statistical analyses were conducted using the R statistical environment[21].

Results

Participants

Two hundred twelve consecutive patients (142 males, 70 females) with a mean age of 59 ± 16 years were admitted to the Department from January 2011 to July 2017 with parotid gland lesions. In thirty-four patients, the medical files were incomplete and did not include FNAC biopsies, and in four patients, permanent histological diagnosis. The results of FNAC biopsy were non-diagnostic in thirteen cases (8 %); the remaining 161 cases (50 females, 111 males) with a mean age of 59 ± 15 years formed the basis of the current study (Figure 1). The sample size was calculated at a minimum number of 137 patients to meet the desired statistical constraints.

Histological results

Most cases (156 patients, 96.8 %) were neoplastic, while the remaining cases (five patients, 3.1 %) were inflammatory. One-fourth of the neoplastic cases (114 patients) were malignant (Table 1). The most common diagnosis was Warthin tumors in 53 patients (34 %), followed by pleomorphic adenomas in 52 patients (33.5 %), primary carcinomas (20 patients, 13 %), and metastasis (19 patients, 12 %) (Figure 2).

Table 1: The 2 x 2 contingency table of fine needle aspiration cytology against permanent histology in differentiating benign from malignant lesions.

	Histology		
FNAC	**Malignant**	**Benign**	**Total**
Malignant	**112**	**3**	**115**
% within FNAC Results	97.4 % (112/115)	2.6 % (3/115)	100 % (115/115)
% within Histology Results	98.2 % (112/114)	7.3 % (3/41)	74.2 % (115/155)
% within Total	**72.3 % (112/155)**	**1.9 % (3/155)**	**74.2 % (115/155)**
Benign	**2**	**38**	**40**
% within FNAC Results	5 % (2/40)	95 % (38/40)	100 % (40/40)
% within Histology Results	1.8 % (2/114)	92.7 % (38/41)	25.8 % (40/155)
% within Total	**1.3 % (2/155)**	**24.5 % (38/155)**	**25.8 % (40/155)**
Total	**114**	**41**	**155**
% within FNAC Results	73.5 % (114/155)	26.5 % (41/155)	
% within Histology Results	100 % (114/114)	100 % (41/41)	**100 % (155/155)**
% within Total	**73.5 % (114/155)**	**26.5 % (41/155)**	

FNAC: fine needle aspiration cytology.

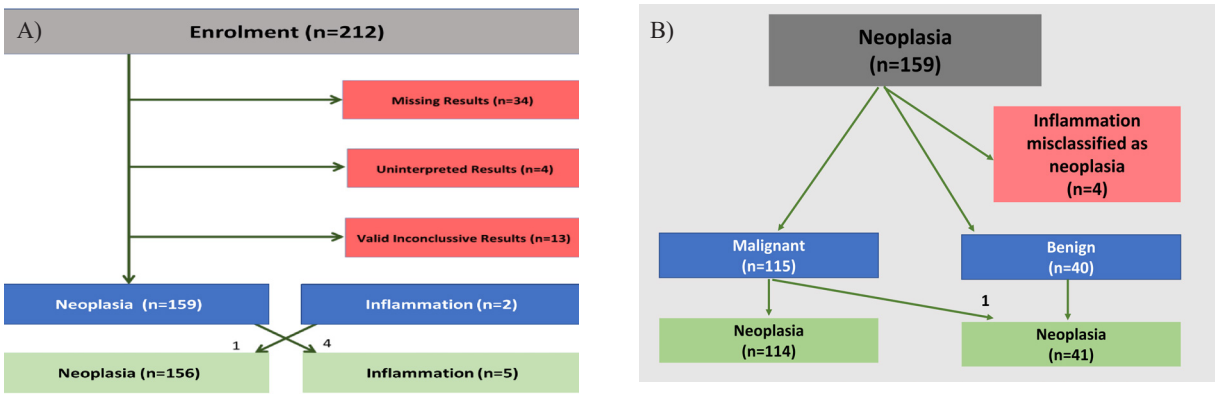

Figure 1: The patient flow chart included in this retrospective case series. Fine needle aspiration cytology (FNAC) results are depicted with blue filing, while histology results are in green filing. A) From a total of 212 patients, there were 34 patients without FNACs, four without permanent histology diagnosis, and thirteen FNACs without a precise diagnosis. FNAC misdiagnosed one neoplastic lesion as inflammatory and four inflammatory lesions as neoplastic. B) The study sample included 114 patients with benign neoplasms and 41 malignancies. FNAC misclassified one patient with a benign lesion as a malignant lesion.
n: number.

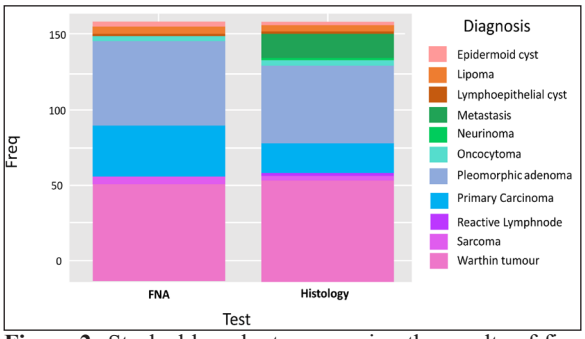

Figure 2: Stacked bar charts comparing the results of fine needle aspiration cytology (FNAC) and histology in diagnosing suspected parotid gland lesions in this retrospective case series.

Freq: frequency, FNA: fine needle aspiration.

FNAC results

The results of the FNAC were compatible with neoplastic disorders in 156 cases (98.75 %), from which 40 cases (25.8 %) had characteristics of malignancy (Figure 3). The diagnosis was pleomorphic adenoma in 55 cases (35.5 %), Warthin tumors in 51 cases (33 %), and primary carcinoma in 39 cases (25 %). Metastatic lesions were largely misdiagnosed as primary carcinomas (Figure 2).

Inflammation vs Neoplasia (Q1)

Overall, 159 parotid gland lesions were initially described as neoplastic by the FNAC. Among them, 155 (97.5 %) were finally diagnosed as neoplastic (true positive) and four (2.6 %) as inflammatory (false positive) by the histology examination. Moreover, two parotid lesions were inflammatory according to the FNAC, whereas the inflammation was eventually histologically confirmed in only one (true negative). Subsequently, the sensitivity and specificity of FNA were calculated to be as high as 50 % and 97 %, respectively (Table 2). Given a positive test result, the post-test probability of neoplasia is as high as 14 %. Moreover, given a negative test result, the post-test probability of an underlying neoplastic lesion is 0.5 % (Figure 3A).

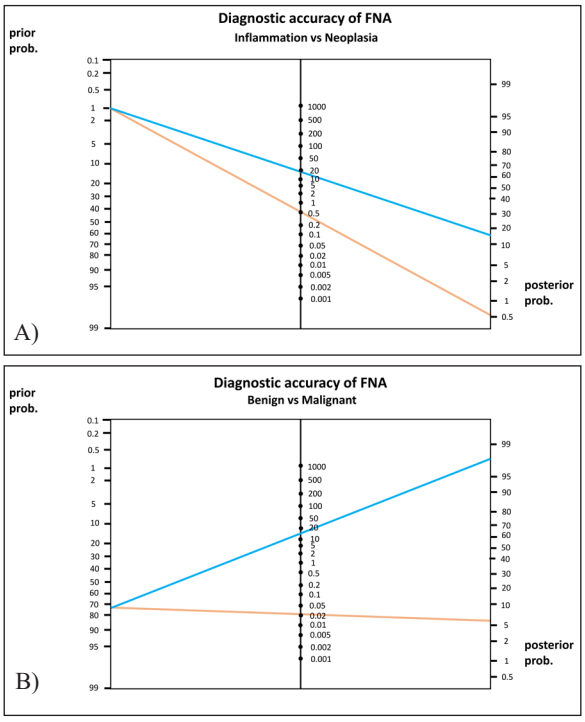

Figure 3: Calculating the post-test probabilities using Fagan's nomogram. A) Inflammation vs Neoplasia. B) Benign vs Malignant lesions.

Prior prob: prior probability, posterior prob: posterior probability

Benign vs malignant lesions (Q2)

The 2 x 2 contingency table of the FNAC and the respective permanent histology in differentiating benign lesions from their malignant counterparts is displayed in Table 1. FNAC's sensitivity and specificity in differentiating malignant lesions from their benign counterparts were as high as 98 % and 93 %, respectively (Table 2). Given a positive test result, the post-test probability of having a malignancy is 98 %. Given a negative test result, the post-test probability of not having a malignant lesion is 6 % (Figure 3B). The detailed results of the diagnostic accuracy parameters are displayed in Table 2 and Figure 4, while Figure 5 analyses the final histology diagnosis of the FNACs' non-diagnostic cases.

Discussion

Parotid gland neoplasms represent about 3 % of head and neck tumours[24]. Whereas most of them, almost 80 %, are benign, performing a meticulous diagnostic workup is essential to verify the neoplasm's nature. FNAC constitutes a necessary part of the preoperative evaluation[12]. With the current study, we attempted to estimate the accuracy of FNAC in differentiating inflammatory parotid gland lesions from neoplasms, and benign from malignant tumors. We recruited 212 patients during an eight-year period that presented to the University Hospital of Larissa with parotid gland lesions. Our study's sample size is comparable to the most extensive and recent studies in the field, along with Dostalova et al (604 FNACs), Galli et al (554 FNACs), Boldes et al (505 FNACs), Alwagdani et al (194 FNACs), Hamour et al (176 FNACs), and Fundakowski et al (317 FNACs)[2,3,9,25]. The composition of the current study's sample was in concordance with the literature, as neoplastic lesions formed the majority of the cases, while inflammatory lesions represented only a minority[9,26,27]. Malignant lesions accounted for approximately a quarter of the gathered cases[3,12,27]. Benign lesions outweighed their malignant counterparts, whereas pleomorphic adenomas and Warthin tumors represented most cases[24-27].

In the presented series, FNAC recorded a moderate sensitivity and high specificity in differentiating neoplastic from non-neoplastic disorders. According to the meta-analysis by Schmidt et al, the summary estimates for the sensitivity and specificity for the differentiation of neo-

Table 2: Point estimates and 95 % confidence intervals of fine needle aspiration cytology. Sensitivity, specificity, disease prevalence, positive and negative predictive value, and accuracy are expressed as percentages. Confidence intervals for sensitivity, specificity, and accuracy are "exact" Clopper-Pearson confidence intervals. Confidence intervals for the likelihood ratios are calculated using the "Log method" as described on pages 109-110 of Altman et al, 2000[22]. Confidence intervals for the predictive values are the standard logit confidence intervals given by Mercaldo et al, 2007[23].

	Neoplastic vs Inflammatory lesions	Malignant vs benign lesions
Apparent prevalence	0.03 (0.01-0.07)	0.74 (0.67-0.81)
True prevalence	0.01 (0.00-0.04)	0.74 (0.66-0.80)
Sensitivity	0.50 (0.01-0.99)	0.98 (0.94-1.00)
Specificity	0.97 (0.94-0.99)	0.93 (0.80-0.98)
Positive predictive value	0.20 (0.01-0.72)	0.97 (0.93-0.99)
Negative predictive value	0.99 (0.96-1.00)	0.95 (0.83-0.99)
Positive likelihood ratio	19.88 (3.67-107.74)	13.43 (4.52-39.92)
Negative likelihood ratio	0.51 (0.13-2.05)	0.02 (0.00-0.07)

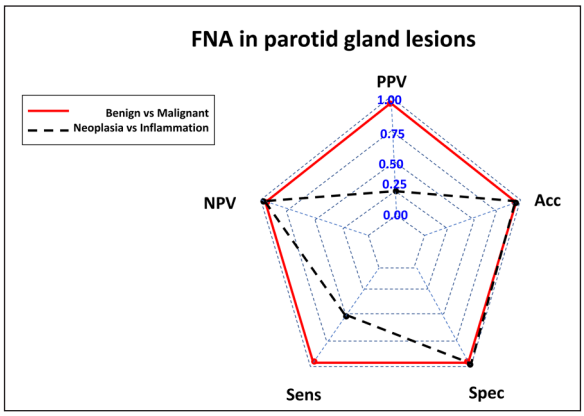

Figure 4: Radar chart of the fine needle aspiration cytology diagnostic accuracy.
FNA: fine needle aspiration, PPV: positive predictive value, Acc: accuracy, Spec: specificity, Sens: sensitivity, NPV: negative predictive value.

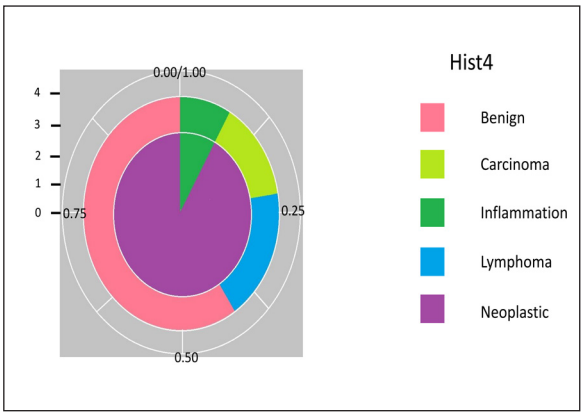

Figure 5: Pie and donut charts on the same plot, of the fine needle aspiration cytology misdiagnosed results. Pie: inflammation vs neoplasia, donut: benign vs malignant lesions.
Hist4: Histopathology result

plastic from non-neoplastic lesions (Q1) were 0.96 [95 % confidence interval (CI): 0.83-0.99] and 0.98 (95 % CI: 0.67-1.00), respectively[28]. Given our series' small number of inflammatory cases, we appreciate that our results should be read cautiously. On the contrary, we observed that the diagnostic accuracy in segregating benign from malignant tumors was extremely high among our cases. We are confident of this result as the number of benign or malignant neoplastic disorders allows us to draw relatively safe conclusions. In the most recent relevant meta-analysis by Liu et al, the summary estimates regarding the sensitivity and specificity of FNAC in differentiating malignant from benign parotid lesions (Q2) were comparable to our results, with 0.78 (95 % CI: 0.740-0.820) and 0.98 (95 % CI: 0.970-0.980), respectively[4]. Similar pertinent findings are reported in the previous meta-analysis by Schmidt et al[28].

The management of salivary gland lesions involves a range of modalities, including information from clinical history and examination, imaging, and cytology or/and histology data[2,29]. The FNAC biopsy has gained popularity as a means of preoperative diagnosis, given that it is relatively straightforward and inexpensive. Thus, multiple case-series studies focusing on reporting the FNAC's efficacy in identifying parotid masses are encountered in the contemporary international literature[2,3,12,13,24,26,27,30]. Copious information concerning its effectiveness in differentiating non-neoplastic vs neoplastic lesions is available. Furthermore, FNAC appears to be rather helpful in discriminating benign from malignant neoplasms[26,27]. Thus, relying on FNAC diagnosis, salivary gland tumors can be triaged and treated according to their urgency, which is highly important since FNAC can guide the decision for surgery and the extent of resection and permits the patient's involvement in treatment decision-making[12]. According to the tumor's histopathologic type, the patient and the surgeon can adequately discuss the management options and choose, if possible, to wait under surveillance instead of undergoing a major operation, which might be particularly useful for older patients with several comorbidities[3,8].

However, it must be acknowledged that FNAC is still considered controversial since, at times, it fails to set a definite diagnosis, considering its elevated rates of false positive and negative results[2]. This is mainly attributed to the vast heterogeneity of the salivary gland pathology

that presents a high percentage of overlapping features. The differentiation between benign and malignant lesions may be challenging primarily because low-grade neoplasms, representing about 50 % of parotid carcinomas, are characterized by less atypia, leading, unfortunately, to their misdiagnosis with benign lesions[2,31]. On the other hand, FNAC is significantly more efficient in identifying benign neoplasms. According to Omura et al[30], FNAC misdiagnosed benign tumors as malignant in only 1.3 %, which is relevant to our result of 1.9 %. This could be attributed to the fact that, since benign neoplasms are much more frequent than malignant ones, cytologists are usually adequately experienced in correctly recognizing them. Considering the operator-dependent nature of the FNAC, immense expertise in this challenging field of head and neck pathology is of great importance for improving management outcomes and morbidity of the patients[11,32]. Specifically, it would minimize the misdiagnosis of carcinomas and the false negative results of specific benign tumors that can transform into malignant ones, such as pleomorphic adenoma[2,11].

Moreover, it is generally accepted that, besides accurate histopathology, correct grading of malignant tumors constitutes a principal component of an accurate diagnosis, since both therapeutic strategy and prognosis differ between high- and low-grade neoplasms. As the study of Suzuki et al mentions[27], FNAC was able to correctly identify both the histopathology and grade of malignant tumors in only 18.9 %. Hence, it is inferred that FNAC's inadequacy constitutes a limitation for its use as a definite diagnostic modality[27]. Last but not least, as highlighted by several studies, the diagnostic efficiency of FNAC is clinically significant only if the non-diagnostic FNAC results are encompassed in the statistical analysis. Excluding these may ostensibly increase the technique's sensitivity rate, but clinicians should remember that this does not correspond entirely to reality[11,27,28]. Galli et al mentioned in their retrospective study that FNAC's sensitivity rate was estimated at 59 % when only the diagnostic results were included in the analysis. In contrast, it decreased at 48 % when the non-diagnostic results were also taken into consideration[11].

The current study has some significant limitations. Firstly, the number of non-neoplastic disorders was small and practically limited to inflammatory disorders. Furthermore, non-surgical candidates were excluded from the study's results due to the inherent nature of the study design, as well as those with non-diagnostic FNAC results. Another potential source of bias that must be acknowledged is the study's retrospective nature. Also, it is worth noting that in clinical practice, the results of FNAC are interpreted in the context of clinical and radiological data. However, in the current study, we only used the results of FNAC.

In conclusion, our series, in accordance with the majority of published data, support the value of FNAC in the management of salivary gland lesions. FNAC is moderately effective in differentiating inflammatory from neoplastic diseases and highly accurate in selecting malignant lesions from benign ones. Although the lack of FNAC sensitivity can occasionally be problematic, it still comprises a valuable tool in salivary gland surgery.

Conflict of interest:
All authors declare no conflicts of interest.

References

1. Gontarz M, Bargiel J, Gąsiorowski K, Marecik T, Szczurowski P, Zapała J, et al. Epidemiology of Primary Epithelial Salivary Gland Tumors in Southern Poland-A 26-Year, Clinicopathologic, Retrospective Analysis. J Clin Med. 2021; 10: 1663.
2. Boldes T, Hilly O, Alkan U, Shkedy Y, Morgenstern S, Shpitzer T, et al. Accuracy, predictability and prognostic implications of fine-needle aspiration biopsy for parotid gland tumours: A retrospective case series. Clin Otolaryngol. 2021; 46: 1065-1072.
3. Alwagdani A, Alhejaili M, Alanzi A, Alghamdi S. Role of fine needle aspiration in malignant parotid tumors at a single-center experience: A retrospective cohort study. Ann Med Surg (Lond). 2021; 64: 102229.
4. Liu CC, Jethwa AR, Khariwala SS, Johnson J, Shin JJ. Sensitivity, Specificity, and Posttest Probability of Parotid Fine-Needle Aspiration: A Systematic Review and Meta-analysis. Otolaryngol Head Neck Surg. 2016; 154: 9-23.
5. Gökçe E, Beyhan M. Advanced magnetic resonance imaging findings in salivary gland tumors. World J Radiol. 2022; 14: 256-271.
6. Pellegrini M, Pulicari F, Zampetti P, Scribante A, Spadari F. Current Salivary Glands Biopsy Techniques: A Comprehensive Review. Healthcare (Basel). 2022; 10: 1537.
7. P A, C A, Masilamani S, Jonathan S. Diagnosis of Salivary Gland Lesions By Fine Needle Aspiration Cytology and Its Histopathological Correlation in A Tertiary Care Center of Southern India. J Clin Diagn Res. 2015; 9: EC07-EC10.
8. Mullen D, Gibbons D. A retrospective comparison of salivary gland fine needle aspiration reporting with the Milan system for reporting salivary gland cytology. Cytopathology. 2020; 31: 208-214.
9. Hamour AF, O'Connell D, Biron VL, Allegretto M, Seemann R, Harris JR, et al. Clinical diagnostic utility of ultrasound-guided fine needle aspiration biopsy in parotid masses. Ear Nose Throat J. 2021: 1455613211058922.
10. Pusztaszeri M, Rossi ED, Baloch ZW, Faquin WC. Salivary Gland Fine Needle Aspiration and Introduction of the Milan Reporting System. Adv Anat Pathol. 2019; 26: 84-92.
11. Galli A, Tulli M, Giordano L, Biafora M, di Santo D, Bondi S, et al. Fine needle aspiration cytology for parotid neoplasms: risk of malignancy through inconclusive results and lower grade tumors. Eur Arch Otorhinolaryngol. 2020; 277: 841-851.
12. Eytan DF, Yin LX, Maleki Z, Koch WM, Tufano RP, Eisele DW, et al. Utility of preoperative fine needle aspiration in parotid lesions. Laryngoscope. 2018; 128: 398-402.
13. Seyhun N, Doğan U, Çalış ZAB, Kaya MF, Hasçiçek SÖ, Turgut S. The role of fine needle aspiration biopsy in deep lobe parotid tumors: Comparison of preoperative cytology and postoperative histopathologic results. Am J Otolaryngol. 2021; 42: 102590.
14. Office of the Assistant Secretary for Planning and Evaluation. Health Insurance Portability and Accountability Act of 1996. Available at: https://aspe.hhs.gov/reports/health-insurance-portability-accountability-act-1996, date accessed: 16/01/2022.
15. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013; 310: 2191-2194.
16. Kim J, Seo BS. How to calculate sample size and why. Clin Orthop Surg. 2013; 5: 235-242.
17. NCCN National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Head and Neck Cancers. Available at: http://www.nccn.org/

professionals/physician_gls/PDF/head-and-neck.pdf, date accessed: 16/01/2022.
18. Woronzoff-Dashkoff KK. The wright-giemsa stain. Secrets revealed. Clin Lab Med. 2002; 22: 15-23.
19. Barnes L, Eveson JW, Reichart P, Sidransky D (eds). World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Chapter 5. Tumours of the Salivary Glands. IARCPress, Lyon, 2005, 209-274.
20. Caraguel CG, Vanderstichel R. The two-step Fagan's nomogram: ad hoc interpretation of a diagnostic test result without calculation. Evid Based Med. 2013; 18: 125-128.
21. The R Foundation. The R Project for Statistical Computing. Available at: https://www.r-project.org/, date accessed: 16/01/2022.
22. Altman DG. Diagnostic tests. Altman DG, Machin D, Bryant NT, Gardner MJ (Eds). Statistics with Confidence. Confidence intervals and statistical guidelines. 2nd Edition. BMJ Books. J W Arrowsmith Ltd, Bristol, 2000, 105-119.
23. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case-control studies. Stat Med. 2007; 26: 2170-2183.
24. Altin F, Alimoglu Y, Acikalin RM, Yasar H. Is fine needle aspiration biopsy reliable in the diagnosis of parotid tumors? Comparison of preoperative and postoperative results and the factors affecting accuracy. Braz J Otorhinolaryngol. 2019; 85: 275-281.
25. Fundakowski C, Castaño J, Abouyared M, Lo K, Rivera A, Ojo R, et al. The role of indeterminate fine-needle biopsy in the diagnosis of parotid malignancy. Laryngoscope. 2014; 124: 678-681.
26. Dostalova L, Kalfert D, Jechova A, Koucky V, Novak S, Kuchar M, et al. Reply to letter to the editor "The role of fine-needle aspiration biopsy (FNAB) in the diagnostic management of parotid gland masses with emphasis on potential pitfalls". Eur Arch Otorhinolaryngol. 2020; 277: 2941.
27. Suzuki M, Kawata R, Higashino M, Nishikawa S, Terada T, Haginomori SI, et al. Values of fine-needle aspiration cytology of parotid gland tumors: A review of 996 cases at a single institution. Head Neck. 2019; 41: 358-365.
28. Schmidt RL, Hall BJ, Wilson AR, Layfield LJ. A systematic review and meta-analysis of the diagnostic accuracy of fine-needle aspiration cytology for parotid gland lesions. Am J Clin Pathol. 2011; 136: 45-59.
29. Wei S, Layfield LJ, LiVolsi VA, Montone KT, Baloch ZW. Reporting of fine needle aspiration (FNA) specimens of salivary gland lesions: A comprehensive review. Diagn Cytopathol. 2017; 45: 820-827.
30. Omura S, Kawata R, Higashino M, Nishikawa S, Terada T, Haginomori SI, et al. Challenges with preoperative diagnosis of low/intermediate-grade carcinoma of the parotid gland: single-center study of 112 patients. Eur Arch Otorhinolaryngol. 2020; 277: 2031-2039.
31. Rossi ED, Baloch Z, Pusztaszeri M, Faquin WC. The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC): an ASC-IAC-sponsored system for reporting salivary gland fine-needle aspiration. J Am Soc Cytopathol. 2018; 7: 111-118.
32. Mantsopoulos K, Bessas Z, Sievert M, Müller SK, Koch M, Agaimy A, et al. Frozen Section of Parotid Gland Tumours: The Head and Neck Pathologist as a Key Member of the Surgical Team. J Clin Med. 2022; 11: 1249.