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Reduced percentage of inconclusive reports and need for repeated fine needle aspirations in ultrasound versus free-hand parotid cytology

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Abstract

Background Fine needle aspiration cytology has been established as a minimally invasive, non-tumour seeding investigation of choice in the initial diagnostic pathway of parotid lesions. The purpose of this study was to compare the accuracy of fine needle aspiration cytology performed with and without ultrasound to determine whether one method should be preferred to the other. A retrospective review of all patients undergoing fine needle aspiration cytology with and without ultrasound for parotid masses in a large district general hospital between 2012 and 2016 was performed. Specificity, sensitivity, accuracy, positive and negative predictive value, percentage of inconclusive fine needle aspiration cytology and percentage of second fine needle aspiration cytology were determined for each group.

Results A total of 397 fine needle aspiration cytology results were available for analysis. The numbers performed with ultrasound guidance and free-hand were roughly equal (208 (52.3%) versus 189 (47.7%)). The number of inconclusive fine needle aspiration cytology reports was significantly higher in the free-hand group (65/189 (34.4%)) than the ultrasound group (25/208 (12%)) (p < 0.0001). A significantly higher number of repeated fine needle aspiration cytology were undertaken in the free-hand group vs ultrasound group (43 vs 15, p < 0.0001); overall 7.2% of ultrasound-guided fine needle aspiration cytology required a second fine needle aspiration cytology, compared to 22.8% in the free-hand group. The sensitivity, specificity, positive and negative predictive values were all higher in the ultrasound group versus the free-hand group.

Conclusions Ultrasound-guided fine needle aspiration cytology is superior to free-hand fine needle aspiration cytology in the investigation of parotid tumours. There is a significant benefit in reducing the number of inconclusive results and repeat fine needle aspiration cytology, and a potential benefit in improving the sensitivity and positive predictive value, when immediate cytology assessment of the sample quality is not performed.

Keywords FNAC, Parotid, Free-hand, Ultrasound, Accuracy, Cytology

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Background

Tumours of the parotid gland constitute approximately 70% of all salivary gland tumours with majority being benign [1, 2]. They can represent up to 50% of all malignancies from salivary glands [2, 3]. Patients usually present to a head and neck clinic with a palpable neck lump although not uncommonly (15%) patients will be referred to the clinic with an incidental finding on imaging [4].



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Fine needle aspiration cytology (FNAC) is the initial investigation of choice and has been shown to be both accurate and safe [5]. Pre-operative diagnosis of a parotid mass, including differentiating between types of benign or malignant neoplasms is clinically useful in helping to plan the correct management [6]. Inconclusive results can jeopardise the management plan. The decision to follow a conservative path, or the extent of parotid or neck surgery required is impossible without reliable cytological or histological diagnosis. In palpable parotid masses FNAC is often performed free-hand but the use of ultrasound (US)-guided FNAC is common and may improve accuracy [5, 7, 8]. Furthermore, radiological features may indicate the diagnosis and can supply synchronous radiological staging of the parotid tumour and cervical nodes [9]. In our unit, the use of US-guided FNAC in parotid lesions has become commonplace and there is trend for head and neck surgeons to perform FNAC in clinic by using US guidance without a designated radiologist [10]. The purpose of this study was to quantify the benefit of US-guided FNAC in parotid tumours, specifically to compare the accuracy of FNAC with and without US guidance.

Methods

A non-experimental cross-sectional study comparing US-guided FNAC in parotid masses versus free-hand FNAC was performed. Patient data was retrospectively collected using the cytology reporting system (including all consecutive FNAC reports from the parotid gland) across three different hospitals within the same district and health board from January 2012 to December 2016 (period of use of both FNAC techniques). The excluded cases were the FNAC cases that were duplicated in the database and the FNAC cases with missing report in the database. To assess the differences between groups, several quality indexes were compared. The index tests included proportion of inconclusive cases (including inadequate or non-diagnostic results and indeterminate results) and the need for secondary FNAC. In these tests, all the patients with available FNAC were used. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were assessed and compared only in patients with confirmed histology. The diagnosis of malignancy was the base for the index test. The reference standard was the histopathology result if available. Pre-assessment of the study using the ethical committee guidelines deemed that this study did not require formal ethical committee assessment. All cytology reports were classified according to "The Milan System for Reporting Salivary Gland Cytopathology" [11]. All FNACs undertaken on clinical or radiological suspicion of a mass in the parotid were included in the study. Free-hand or non-guided FNAC was done by consultant head and neck surgeons, US-guided FNAC was done by consultant radiologists. The criterion to perform free-hand nonguided FNAC was the clinical size of the mass that was palpable. FNAC was performed utilising a 23-gauge needle attached to 10 mL syringe with or without aspiration. A minimal of 2 passes were performed either by the surgeon or the radiologist. No cytologist or pathology technician were available at the time of the FNAC to check for sample quality. The obtained aspirates were spread onto glass slides, air dried and later stained with May Grunwald-Giemsa (MGG). The rest of the material was processed in a cytoblock. Patient demographics were collected including age, gender, cytology report, pathology report, and US report. Age was calculated between date of birth and day of cytology request. Grouped and separated index test are given for FNAC performed free-hand or US-guided. Z test was used to compare the difference in proportion of inconclusive reports of US versus freehand FNAC. Confidence interval 95% of the difference was calculated as well as significance and power. Indeterminate cases of FNAC were removed from accuracy analysis and included with inadequate cases to inconclusive cases. Recommendations from the Standards for Reporting of Diagnostic Accuracy Studies (STARD) have been followed [12]. The indeterminate results for the reference standard will be discussed. Intended sample of FNAC to compare proportion of inconclusive reports in US- versus free-hand-guided FNAC was 438 (219 each group) for two tail analysis with an expected difference of 0.1 (10%), alfa = 0.05 and power of 0.8 with an allocation ratio of 1:1 and smaller proportion larger than 10%. Posthoc study has been performed to compare if free-hand FNAC generates more secondary FNAC than US-guided FNAC. Bonferroni correction has been applied. STATA 15.0 statistical software and EXCEL (Redmond, USA) has been used. No blinding was used for collection of data or statistical assessment.

Results

There were 429 reports available from which 17 were duplicated and 15 were missing on the system and could not be accessed. A total of 397 FNAC results were used for this study from which 208 were US-guided FNAC and 189 were performed free-hand in clinic. From the US-guided cases 12.7% were positive for malignancy and from the free-hand FNAC 17.0% were malignant. The percentage of inconclusive US-guided reports was 12.0% (8.7% inadequacy and 3.4% indeterminate) whereas for free-hand FNAC this was 34.4% (26.5% inadequacy and 7.9% indeterminate). Within the negative results for malignancy on the FNAC, 33.3% had confirmed histology available (33.5% US and 33.0% free-hand). Within

the positive results for malignancy on the FNAC, 52.9% had confirmed histology available (50% US and 55.5% free-hand). Within the inconclusive results on the FNAC, 42.2% had confirmed histology available (40% US and 43.1% free-hand). A STARD (Standards for Reporting of Diagnostic Accuracy Studies) flow diagram has been added (Fig. 1) [12].

Among the 397 FNAC, 183 were female (46.9%, 60.1(16.5) years)), of which there were 102 US-guided FNAC (49.0%, 60.5(16.4) years) and 81 free-hand FNAC (42.9%, 59.6(16.8) years). Two hundred fourteen were male (53.1% 61.7(16.1) years), of which there were 106 US-guided FNAC (51%, 64.7(15.1) years) and 108 free-hand FNAC (57.1%, 58(16.5) years). Overall age range for all the patients was 18 to 97 years old.

A total of 182 reports had FNAC with benign diagnosis without confirmed histology available. The most common benign diagnosis from these was Warthin's tumour (34.6%) followed by pleomorphic adenoma (31.9%). On the other hand, 16 reports had FNAC with a malignant diagnosis without confirmed histology available. The most common diagnosis from these were squamous cell carcinoma (SCC, 18.8%) and adenocarcinoma (18.8%, Table 1).

A total of 91 reports had subsequent confirmed histology after a negative FNAC for malignancy. The most common diagnosis was pleomorphic adenoma (47.2%) followed by Warthin's tumour (27.5%). Eighteen reports had subsequent confirmed histology after a positive FNAC for malignancy. The most common diagnosis

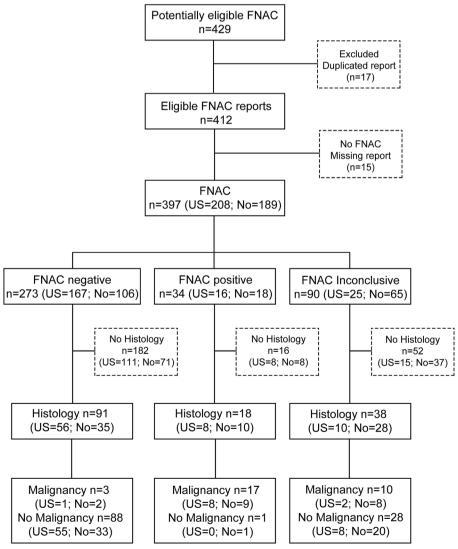


Fig. 1 STARD diagram reporting flow of participants through the study

Table 1 Cytologic diagnosis of parotid gland tumours without histopathologic diagnosis

| | n | % |
|----------------------------------|----|------|
| Benign ($n = 182$) | | |
| Warthin's tumour | 63 | 34.6 |
| Pleomorphic adenoma | 58 | 31.9 |
| Cyst | 19 | 10.4 |
| Benign lymphoid tissue | 13 | 7.1 |
| Benign salivary tissue | 9 | 4.9 |
| Abscess | 6 | 3.3 |
| Oncocytoma | 4 | 2.2 |
| Multiple benign diagnosis | 4 | 2.2 |
| Chronic sialadenitis | 3 | 1.6 |
| Monomorphic adenoma | 1 | 0.5 |
| Metaplastic squamous reaction | 1 | 0.5 |
| Branchial cyst | 1 | 0.5 |
| Malignant ($n = 16$) | | |
| Squamous cell carcinoma | 3 | 18.8 |
| Adenocarcinoma | 3 | 18.8 |
| Poorly differentiated carcinoma | 2 | 12.5 |
| Mucoepidermoid carcinoma | 2 | 12.5 |
| Mucoepidermoid carcinoma vs SCC | 2 | 12.5 |
| Lymphoma | 1 | 6.3 |
| Carcinoma ex-pleomorphic adenoma | 1 | 6.3 |
| Lymphoma vs small cell carcinoma | 1 | 6.3 |
| Multiple malignant diagnosis | 1 | 6.3 |

was squamous cell carcinoma, representing 33.3% of the reports. Finally, 38 reports had subsequent confirmed histology after an inconclusive FNAC. The most common benign diagnosis was Warthin's tumour (23.7%), and the most common malignant diagnosis was mucoepidermoid carcinoma (5.3%). A total of 4 report (3 patients) had an indeterminate histology but all of them were benign. One histology differential diagnosis was between basal cell adenoma and pleomorphic adenoma, another was between pleomorphic versus monomorphic adenoma and a further one was between normal parotid tissue and a cystic lesion not viable for analysis. Duplicate FNAC with same histology were removed for the final histopathologic diagnosis of parotid gland tumours (Table 2).

A total of 139 definitive histology was obtained from 307 patients (45.2%). Of these, 121 (88%) had superficial parotidectomy or extracapsular dissection, 10 had an open biopsy (7%), 2 (1%) had core biopsy, and 6 (4%) had histology of the primary tumour (metastasis). The overall median time between FNAC and the availability of histology was 107.5 days, 132.5 days for US-guided FNAC and 78 days for free-hand FNAC. The time to obtain the definitive histology varied depending on the FNAC results. For negative FNAC the median was

 Table 2
 Histopathologic diagnosis of parotid gland tumours

| | n | % |
|----------------------------------|----|------|
| Benign (n = 113) | | |
| Pleomorphic adenoma | 50 | 44.2 |
| Warthin's tumour | 33 | 29.2 |
| Benign lymphoid tissue | 5 | 4.4 |
| Indeterminate | 3 | 2.7 |
| Basal cell adenoma | 3 | 2.7 |
| Oncocytoma | 3 | 2.7 |
| Benign salivary tissue | 3 | 2.7 |
| Chronic sialadenitis | 2 | 1.8 |
| Lipoma | 2 | 1.8 |
| Tuberculosis | 2 | 1.8 |
| Epidermal cyst | 1 | 0.9 |
| Monomorphic adenoma | 1 | 0.9 |
| Nodular fasciitis | 1 | 0.9 |
| Lymphoepithelial cyst | 1 | 0.9 |
| Schwannoma | 1 | 0.9 |
| Granulomatosis | 1 | 0.9 |
| Cyst | 1 | 0.9 |
| Malignant ($n = 26$) | | |
| Squamous cell carcinoma | 9 | 34.6 |
| Lymphoma | 6 | 23.1 |
| Melanoma | 5 | 19.2 |
| Mucoepidermoid carcinoma | 2 | 7.7 |
| Carcinoma ex-pleomorphic adenoma | 1 | 3.8 |
| Acinic cell carcinoma | 1 | 3.8 |
| Merkel cell carcinoma | 1 | 3.8 |
| Adenoid cystic carcinoma | 1 | 3.8 |

132 days with interquartile 25% (IQ25) of 80.3 days to interquartile 75% (IQ75) of 247 days. For inconclusive FNAC, the median (IQ25-IQ75) was 96 days (63–188). Finally, for positive FNAC, the median (IQ25-IQ75) was 45 days (29–64).

The main analysis showed that the proportion of inconclusive FNAC (inadequate sampling or indeterminate results) using US (12.0%) was significantly smaller than doing free-hand FNAC in clinic (34.4%). The difference in proportion was 22.4 (CI 95%: 14.2 to 30.3), p < 0.0001. Power was 0.99 for a significance alfa p = 0.05.

To perform the comparison between the different accuracy measures, only 109 patients were used. As seen in the flow diagram, from the 397 FNAC cases the study only included 109 cases, those with available histology and positive (n=91) or negative (n=18) FNAC. The results from diagnostic accuracy measures seem similar whether US or free-hand was used. The sensitivity of US-guided FNAC trended higher (89%) than for free-hand FNAC (82%), whereas specificity seemed more similar (100% and 97%, respectively) (Table 3).

Table 3 Measures of diagnostic accuracy and inconclusive proportion among parotid tumours

| | Total FNAC ($n = 109$) | US-guided FNAC ($n = 64$) | Free-hand FNAC ($n = 45$) |
|-----------------------------|--------------------------|-----------------------------|-----------------------------|
| Sensitivity | 85.0 (62.1–96.8) | 88.9 (51.8–99.7) | 81.8 (48.2–97.7) |
| Specificity | 98.9 (93.9-100) | 100 (93.5–100) | 97.1 (84.7–99.9) |
| Positive predictive value | 94.4 (72.7–99.9) | 100 (63.0–100) | 90.0 (55.5–99.7) |
| Negative predictive value | 96.7 (90.7–99.3) | 98.2 (90.4–99.9) | 94.3 (80.8–99.3) |
| Accuracy | 96.3 (90.9–99.0) | 98.4 (91.6–100) | 93.3 (81.7–98.6) |
| Inconclusive ^a | 22.7 (18.6–27.1) | 12.0 (7.9–17.2) | 34.4 (27.6–41.6) |
| Secondary FNAC ^a | 14.6 (11.5–18.4) | 7.2 (4.4–11.6) | 22.8 (17.4–29.2) |

All values presented in % (95% confidence interval)

Our study also considered the number of secondary FNAC requests after an inconclusive initial FNAC in the two groups. Secondary FNAC was defined as repeated FNAC that was requested 6 months after first FNAC in the same patient. A post-hoc study to assess the difference in proportion of secondary FNAC between USguided and free-hand FNAC was performed. Bonferroni correction significance was set to 0.025. From the 273 FNAC that had a negative result for malignancy, 6 USguided FNAC and 11 free-hand FNAC had another FNAC performed subsequently. From the 34 FNAC that had a positive result for malignancy, only 1 US-guided FNAC and 3 free-hand FNAC had another FNAC performed. Finally, from 90 inconclusive FNAC, 8 USguided FNAC, and 29 free-hand FNAC had another FNAC performed. Overall, US-guided FNAC generated 15 repeated FNAC from a total of 208 and free-hand FNAC generated 43 repeated FNAC from a total of 189. The proportion of secondary repeated FNAC using USguided FNAC (7.2%) was smaller than doing free-hand FNAC in clinic (22.8%). The difference in proportion was 15.5 (CI 95%: 8.6 to 22.6), *p* < 0.0001.

Discussion

Our study comprises one of the largest consecutive series of FNAC reports for parotid tumours and provides an insight into a common first line investigation for parotid masses in head and neck units in this country. The results demonstrate that free-hand or palpation-guided FNAC is acceptably accurate but has a higher inconclusive rate, therefore their usefulness remains questioned. The accuracy of parotid FNAC with or without US (without immediate cytology assessment) has been reported in single studies but not described separately within the same study; combining results from US-guided and free-hand techniques is a problem given the differences this study has shown [5, 13–16]. The difference in accuracy for free-hand versus ultrasound-guided FNAC of parotid masses has been documented in a previous meta-analysis

[5]. The meta-analysis results showed a sensitivity of 0.78 (95% CI, 0.74–0.78) for all FNAC groups that is comparable to sensitivity of 0.82 in the current study. The US group sensitivity 0.84 (95% CI, 0.76–0.91) also compares to 0.89. The meta-analysis specificities again were comparable and they are close to 1. In our study, we have failed to demonstrate a significant difference between the accuracy of US-guided and free-hand FNAC, although a trend can be seen in all measures. This is to be expected since the sample of 109 patients is too small to determine significance for small percentage differences.

The rate of inconclusive and repeat FNAC is significantly higher in free-hand FNAC than when it is performed under US guidance. The inconclusive percentage difference comparing free-hand versus USguided FNAC, without immediate cytology assessment of the sample quality, has been previously documented in a single study for head and neck masses, but no specific studies were found in relation to parotid masses [17]. The study showed similar percentage of inconclusive results with a 33.5% for free-hand versus 34.4% as noted in the present study and 15.3% for US-guided FNAC versus 12% as noted in the present study. In the comparing study, the percentage of free-hand inadequate specimens was 21.5% compared to 26% in the present study whereas the percentage of free-hand indeterminate samples was 12% compared to 7.9%. In the reference study, the US-guided FNAC inadequacy percentage was lower (3.4%) than in the current study (8.7%) whereas the indeterminate samples percentage was higher (11.9% versus 3.4%). The use of US reduced the inadequacy (21.5 to 3.4) whereas it did not affect the percentage of indeterminate samples (12 to 11.9) in the comparing study when using US-guided FNAC. Conversely, the present study showed both reduction in inadequacy (26.5 to 8.7) and indeterminate samples (7.9) to 3.4) when using US-guided FNAC. It is plausible that US use could improve both inadequacies and indeterminate samples in parotid masses.

^a Total FNAC n = 397 (US = 208; No = 189)

The percentage of inadequacy and indeterminate samples has been documented recently in a study with immediate assessment of the FNAC sample. The percentage of inadequacy was similar in free-hand versus US-guided FNAC (11 to 12). Likewise, the percentage of indeterminate samples was similar as well (4 to 6). In this situation, using ultrasound with FNAC would become beneficial when there is no immediate assessment of the sample quality [18]. Likewise, another recent study has shown that ultrasound-guided FNAC by the same surgeons without immediate cytology assessment had lower inadequacy rate than cytopathology free-hand FNAC with immediate sample assessment (3 to 7.2). In this last study, no information was provided regarding indeterminate samples [10]. The current study supports this hypothesis. The rate of inadequacies has been reported in a previous meta-analysis for all but not specific for free-hand or US-guided FNAC. The overall results for this metaanalysis showed 5.3% percentage of non-diagnostic or indeterminate samples which is higher than US-guided FNAC (3.4%) and lower than free-hand FNAC (7.9%) in the current study. Likewise, inconclusive results account for 14.7% percentage of the meta-analysis which is higher than US-guided FNAC (12%) and lower that free-hand FNAC (34.4%). However, it needs to be considered that when doing meta-analysis there is a lack of good information about non-diagnostic and inconclusive reports as documented by the same meta-analysis which could be selecting the best studies and biasing the actual results [5].

The reasons for the difference between free-hand and US-guided FNAC are not well documented in the literature. They may include operator experience and pathologist experience (if it is not the operator) [19]. It is known that the inadequacy percentage can be related to the presence of a one-stop service. A recent systematic review for head and neck FNAC has demonstrated the benefit of the service [20].

The importance of an abnormal rate of inconclusive FNAC results is that it seems to be related to an increased risk of malignancy as documented in previous studies [8, 21]. In this study, there was an incidence of 11% in malignant tumours after an inconclusive FNAC report and as such it is recommend all inconclusive FNAC reports be treated with an appropriate index of suspicion.

The use of US-guided core needle biopsy is an alternative technique to consider since it can increase the sensitivity to 0.96 with specificity of 1. However, it comes with increased risk of facial hematoma (1.6%), facial nerve weakness (0.2%) and a possibility of seeding [22, 23].

To define the limits of the present study, STARD recommendations have been followed. QUADAS assessment guidelines have been helpful as well [12, 24, 25].

The description of FNAC process can introduce bias to the study since more than 10 surgical clinician and 3 radiologists have been involved with FNAC over the studied period. This problem, undoubtedly, increases variability of the described FNAC procedure [12, 19, 26].

Selection bias is likely to have happened when referring patient for US-guided FNAC. It is expected that patients with difficult clinical assessment or consultants with less experience in FNAC have referred more patients for US-guided FNAC while patients with suspected malignancy have had clinical FNAC to reduce time to get final diagnosis. Malignancy rate provides some light to the possible bias. In the study, 12.7% US-guided cases were positive for malignancy and 17.0% free-hand FNAC were malignant. According to these results, some bias can be expected that could minimise the difference found [12, 19, 26].

Selection bias could have happened as well since only 45.2% (139/307) of patients with FNAC eventually had available histology results by the first half of 2017. Some of these patients may have had some contraindications for surgery and others may have wished to have further radiological and clinical follow up instead of having the excision. That seems to be a common situation in parotid and head and neck studies including large series of around one thousand FNAC ranging from 28.6 to 52.8% [27-30]. Moreover, it is common not to report the amount of total FNAC performed or to include only the patients that have undergone surgery, excluding those that had the FNAC without surgery [4, 10, 14–16, 18]. This situation seems to be critical since there is rarely documentation regarding what were the results of the FNAC that did not have surgery or histology. In the present study, all FNAC have been documented to compensate for this bias (Table 1). It would be recommended that parotid cytology studies include any results of FNAC without histology as a quality marker.

Finally, this study was retrospective which could have introduced some bias by not selecting cases that had US and the US was enough to reduce need for FNAC whereas in the clinical setting this information may be missing unless there is a previous US report available. Verification bias affects the current study as with all, particularly retrospective FNAC studies since malignancies have more confirmed histology available than benign neoplasms. Review bias affects the study since it was not blinded. Misclassification bias is expected at a rate of 3% [12, 19, 26].

The median time to obtain the confirmed histological diagnosis was 55 days faster (78 vs 132.5) for the free-hand group compared to the US group. Since most parotid masses turn out to be benign the referral pathway preferring urgent, rather than suspected cancer

might explain this delay. The increased percentage of malignancy within free-hand group (17 vs 12.7) and the increased inconclusive rate (34.4 vs 12) could have prompted histology being available more readily in the free-hand group [12, 19, 26].

Although it is not recommended free-hand FNAC for parotid lesions based on these results, US-guided FNAC may not be readily available in some clinics. In these examples, the benefit of free-hand FNAC is to reduce the time to obtain the cytology. The knowledge of FNAC technique whether US-guided or not is a valuable skill for the head and neck specialist, and it should be a standard for trainees [10]. The recent proliferation of US FNAC instructional courses aimed at non-radiology trained practitioners suggest that in the future it may be more common for US FNAC to be performed by the surgeon in the head and neck clinic, should a designated head and neck radiologist not be available. However, the expert head and neck radiologist is invaluable in describing the mass features such as suspicious malignancy and probable diagnosis that are relevant to the management of the patient independently from FNAC outcome. Clearly, the gold standard would be a one stop US FNAC neck lump clinic and option to core biopsy with both radiologist and cytologist in attendance, but such clinics are unfortunately not the norm in most national centres. We hope this study helps the planification of equipment in geographic area where there is more prevalence of parotid pathology [7, 31, 32].

Conclusions

Free-hand FNAC is a safe procedure with comparable results to US-guided FNAC and it is still used to reduce cytology report delays. US-guided FNAC significantly reduces the number of inconclusive results and repeat FNAC compared to free-hand FNAC, when immediate cytology assessment of the sample is not performed. The US FNAC neck lump clinic with immediate assessment of the sample is still the gold standard, but it is not feasible in many departments. Further training of surgeons to perform US may increase the use of US-guided FNAC in the head and neck clinic, reducing time for cytology diagnostic.

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Code availability

Code for STATA available on request from the corresponding author.

Authors' contributions

Conceptualization: AKA. Methodology: EP, AKA and MZM. Data Collection: IGR. Formal analysis and investigation: EP. Writing – original draft preparation: EP. Writing – review and editing: ASH, MZM, HZ and AKA. Supervision: MZM and AKA. All authors read and approved the final manuscript.

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Availability of data and materials

Original anonymised and encrypted data (Excel) is available from the corresponding author.

Declarations

Ethics approval and consent to participate

No ethical approval is required for retrospective data from the care team under NHS policy. According to the NHS tool from the Medical Research Council, NHS REC review for sites in Wales was not required. Consent to participate was not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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