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ORIGINAL ARTICLE

Ultrasound-guided fine-needle capillary cytology of parotid gland masses coupled with a rapid-on-site evaluation improves results

La ponction cytologique écho-guidée de la glande parotide couplée à l'examen direct sur place améliore les résultats

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KEYWORDS
Ultrasound-guided fine-needle cytology;
Parotid gland;
Direct-on-site evaluation

Summary
Objective of the study. — To test whether a direct-on-site microscopic examination of fresh, unstained puncture slides by the radiologist decreases the rate of false-negative cases on ultrasound-guided fine-needle cytology of parotid gland masses.
Patients. — Thirty parotid gland masses from 28 patients were punctured under ultrasound guidance. The same group was used as its control group.
Methods. — After one or two passes, the material was spread on slides and air-dried (control group, without microscopic examination). For the study group, it was thus analyzed unstained under the microscope. A sample was considered adequate if at least six clusters of parotid cells were found per slide on at least two slides. For the study group, new punctures were obtained and slides prepared until this condition was fulfilled.

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Results. — Of the 30 evaluated masses, 100% benefited from a cytological diagnosis after microscopy. Twenty-four were adequate in the control group, while 30 were adequate in the study group. The maximum number of punctures to obtain an adequate sample was six. On-site direct microscopy significantly increased the number of adequate specimens by 20% ($P = 0.03$, CI [1.63—20%]).
Conclusion. — Direct and systematic examination of slides by a radiologist avoided the risk of false-negative results caused by having insufficient sample material.

Résumé
Objectif de l'étude. — Évaluer si l'examen microscopique direct à l'état frais par le radiologue de lames de cytoponctions écho-guidées parotidiennes diminue le taux de faux-négatifs.
Patients. — Trente tumeurs parotidiennes provenant de 28 patients ont fait l'objet d'une cytoponction écho-guidée. Le même groupe de patients a servi de témoin.
Méthodes. — Après un ou deux passages à l'aiguille, le matériel était étalé sur lames et séché à l'air (groupe témoin, sans examen microscopique direct). Pour le groupe expérimental, le matériel était examiné au microscope sans coloration. Un échantillon était considéré comme adéquat si au moins 6 amas cellulaires étaient présents par lame sur au moins deux lames. Dans le groupe expérimental, de nouvelles ponctions étaient réalisées si ce critère n'était pas rempli jusqu'à son obtention.
Résultats. — Sur les 30 tumeurs prélevées, 100 % ont bénéficié de l'examen microscopique direct. Vingt-quatre ponctions étaient satisfaisantes dans le groupe témoin et 30 dans le groupe expérimental. Le nombre maximal de ponctions pour obtenir du matériel satisfaisant était de six. L'examen direct sur place a augmenté de 20 % le nombre d'échantillons satisfaisants pour évaluation ($p = 0{,}03$, IC [1,63—20 %]).
Conclusion. — L'examen microscopique direct systématique des lames de cytoponction par le radiologue évite le risque de faux-négatifs liés à un matériel insuffisant.

Introduction

To date, MRI and fine-needle cytology (FNC) represent the best methods to evaluate parotid gland masses and to plan surgery [1]. FNC is a reliable diagnostic technique in the hands of an experienced cytopathologist [2,3], even for pediatric parotid tumors [4,5]. The sensitivity of diagnosis of malignant lesions is high: up to 93%, although the sensitivity of identification of tumor type is lower [6]. FNC enables a cytological diagnosis in most cases; the method is minimally invasive and is easy to perform. Preoperative FNC diagnosis improves the surgical outcomes of parotid masses [1,7]. However, the most significant problem with FNC is that the procedure frequently obtains inadequate material, thus making evaluation impossible [8,9].

Studies confirm the high overall accuracy of evaluating parotid masses using fine-needle aspiration ranging from 90—98% [10—12]; however, unsatisfactory aspirates (poorly cellular or blood-only aspirates) can occur in up to 18% of cases [13,14]. This occurs even if the adequacy of the sample itself is improved using an ultrasound (US)-guided biopsy [12,15,16].

Repeated FNCs may provide a cytological diagnosis in cases where the initial diagnosis is unclear [2], but delays in diagnosis represent an additional source of pain and anxiety for the patient. Moreover, evidence suggests that the first aspiration may modify the lesion, thus hampering further interpretation. Indeed, although a rare event, FNC can lead to hemorrhage or cellulitis at the needle-puncture site [17], and may transform a simple Warthin's tumor into a metaplastic variant [18]. Furthermore, difficulties in interpreting the great diversity of histologic subtypes of salivary-gland neoplasms, especially if they are malignant [11,19], may lead to errors in diagnosis in many cases because of limited sampling. Thus, there is great added value if the operator achieves successful aspiration and can send the cytologist adequate samples that have already been verified.

In our institution, 30% of parotid gland cytologies were inadequate when the puncture was performed without US guidance. We thus decided to use US guidance, and the rate decreased to 20% (unpublished data). However, as a rapid-on-site direct examination may improve results, we decided to train the radiologist himself to perform microscopic examination. We conducted a study to test whether, by estimating every slide microscopically without staining at the patient's bedside, and to repeat this process until the sample was adequate to obtain a cytological diagnosis, could decrease the number of non-significant samples. In our procedure, we combined the use of a US-guided non-aspiration technique and rapid-on-site evaluation of specimens by the radiologist.

Patients and methods

We conducted a prospective study. We included patients addressed form the ORL department, presenting with a parotid mass, especially when it was deeply located or when

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a previous puncture was inadequate. Exclusion criteria were represented by acute inflammatory masses, when a previous puncture was performed less than 5 days ago or when the patient did not consent. Between January 2013 and August 2014, 28 patients (9 males, 19 females) were included. Thirty masses were examined consecutively as one patient had three parotid masses. Three patients had already undergone a FNC at another center and the results have been described as inadequate. Investigations were carried out according to the Declaration of Helsinki. Each patient was informed about the procedure before the puncture and gave his/her oral consent.

Needle-puncture technique

All FNCs were obtained in our center using local asepsis. Two radiologists, respectively with a two- and four-years of experience, performed the punctures. Because the use of a small-gauge needle (which is less painful) does not reduce the number of acellular aspirations in head-and-neck FNCs, the FNC procedure was performed using a 25-mm 25-gauge needle under the US guidance with a 10—5.5 scan-head (Toshiba, France) [20]. After introducing the needle into the mass, it was moved upwards and downwards for a few seconds. Cytological material was then collected by capillarity without aspiration to avoid a hemorrhagic sample. A syringe containing 10 mL of air was then attached to the needle and the collected material was expelled and smeared carefully onto glass slides that were free of fixation or crushed artefacts. The samples contained minimal blood to ensure that epithelial cells could be readily visualized, without any features to indicate cellular atypia. The sampler underwent two systematic passes (control group). After air-drying, the slides were checked using microscopy by the radiologist to ensure there was adequate parotid material.

Direct microscopic examination and cytological criteria for adequacy

The radiologist performed the microscopic examination after being trained by an experienced cytologist. Direct unstained samples were examined to preserve the material for further cytological stains or ancillary techniques. The rapid RAL555 stain (RAL diagnostics, Martillac, France) was first tested as an aid for cell identification. However, this method was abandoned because of unsatisfactory staining in some cases and difficulty in obtaining further cytological characterization. There are no generally accepted criteria for cellular adequacy. Thus, in agreement with the cytologist, the material was considered adequate if at least six parotid-cell clusters were observed per slide on at least two slides (as it is established for thyroid cytopathology). Examination of the cells was performed without staining, and by removing the condenser to increase cell contrast.

Puncture of the mass was satisfactory if adequate smears were obtained from at least one puncture. Thus, specimen adequacy was assessed after each pass. If adequacy was not obtained, the puncture was repeated without limiting the number of passes. We checked each new sample microscopically until a satisfactory slide was obtained. All cytological specimens were then sent to the laboratory to be interpreted by a trained cytologist. In this study, we considered two groups: ''positive'' patients that required only one or two punctures to obtain a satisfactory sample, and ''negative'' patients where samples were negative after two passes and, thus, would have been inadequate if we had not checked the slides microscopically and repeated the needle punctures until obtaining at least one satisfactory sample.

Statistical analyses

We used the McNemar test to compare the paired samples (on line BiostaTGV).

Relationships between cytology and histopathology

Correlations between cytology and final histopathological diagnoses were examined when both were available.

Results

Puncture procedure under US guidance

The FNC procedure was generally well-tolerated by patients, except for five patients where site of the puncture caused pain: we were then obliged to use only a single sample, although each of these five cases was considered ''positive''. There were no immediate complications after the punctures. At the end of the examination, US guidance allowed the route of the needle in the lesion to be detected: no hematomas were visible (Fig. 1).

Adequacy of the specimens during the FNC procedure and the final cytological diagnosis

The maximum number of passes was six. Thus, as a result of on-site assessment of specimen adequacy, there was 20% repetition of needle punctures required for six masses. Final adequate specimens were obtained for all the masses (100%) after performing additional punctures, and for each sample, a final cytological diagnosis was obtained (Table 1). Samples were considered positive when at least six clusters of cells were present per slide on at least 2 slides.

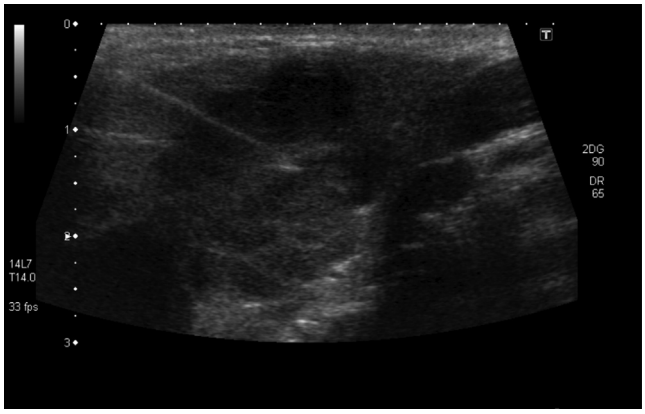

Figure 1 FNCC of a parotid gland under ultrasound guidance. The fine needle is seen within the tumor (on the upper left).

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Table 1 Clinical data, number of passes, cytological and histological diagnoses.

Case number	Gender	Age	Passes	Cytological diagnosis	Histological diagnosis
1	F	23	2	Pleomorphic adenoma	Pleomorphic adenoma
2	F	71	2	Pleomorphic adenoma	NP
3	M	34	2	Oncocytic tumor	Pleomorphic adenoma with oncocytic metaplasia
4	F	64	2	Suspicion of Warthin's tumor	NP
5	F	49	3	Normal lymph node	NP
6	M	65	2	Pleomorphic adenoma	Pleomorphic adenoma
7	F	70	2	Adenoid cystic carcinoma	Adenoid cystic carcinoma
8	M	61	2	Warthin's tumor	Warthin's tumor
9	F	70	2	Pleomorphic adenoma	NP
10	F	65	2	Pleomorphic adenoma	Pleomorphic adenoma
11	F	65	2	Pleomorphic adenoma	Pleomorphic adenoma
12	F	65	2	Pleomorphic adenoma	Pleomorphic adenoma
13	M	51	2	Pleomorphic adenoma	Pleomorphic adenoma
14	F	59	2	Pleomorphic adenoma	Pleomorphic adenoma
15	F	50	3	Suspicion of Warthin's tumor	Low-grade mucoepidermoid carcinoma
16	F	79	2	Acinic-cell carcinoma	Acinic-cell carcinoma
17	F	50	1	Pleomorphic adenoma	Pleomorphic adenoma
18	M	32	2	Lymphoma	Hodgkin's lymphoma
19	M	60	3	Warthin's tumor	Warthin's tumor
20	M	58	3	Warthin's tumor	Warthin's tumor
21	F	54	6	Warthin's tumor	NP (confirmed by another cytology)
22	F	22	5	Suspicious for Warthin's tumor	NP
23	F	82	2	Low-grade mucoepidermoid carcinoma	Low-grade papillary cystadenocarcinoma
24	M	77	2	Follicular lymphoma	Follicular lymphoma
25	F	60	2	Suspicion of basal-cell carcinoma	Adenoid cystic carcinoma
26	M	53	2	Pleomorphic adenoma	Pleomorphic adenoma
27	F	61	2	Pleomorphic adenoma	Pleomorphic adenoma
28	F	34	2	Pleomorphic adenoma	Pleomorphic adenoma
29	F	42	2	Warthin's tumor	Warthin's tumor
30	F	52	2	Pleomorphic adenoma	Pleomorphic adenoma

NP: not performed.

In the background, red blood cells appeared as refringent round cells with regular plasma membranes, whereas the cells of interest appeared as less refringent, larger nucleated cells (Fig. 2). The fibrillar extra-cellular matrix could sometimes be identified in pleomorphic adenomas. The five cases that had a single puncture were realized and were adequate. Furthermore, from samples that had had an inadequate FNC taken in other centers, the results turned out to be satisfactory.

Puncture results

In our study, we examined 30 parotid masses microscopically. We obtained 24 ''positive'' samples after one or two punctures (80%), and 6 ''negative'' samples (20%) required additional passes (at least 3 passes) until they were declared ''satisfactory''. This would not have been possible without direct microscopic examination. Among the 6 ''negative'' results, four masses required 3, one mass required 5, and one mass required 6 punctures. McNemar's test detected statistically significant differences. Direct microscopic examination of the slides and repeated punctures increased the accuracy by 20% ($P = 0.0313$, 95% CI: 1.63—20%). The use of microscopy thus significantly increased the probability of obtaining an adequate sample for cytological analysis.

Final cytological diagnosis

A cytological diagnosis was obtained for each mass (Table 1). The series consisted of 14 pleomorphic adenomas (47%), 8 Warthin's tumors (27%), 1 oncocytic-cell tumor (3%), 2 lymphomas (7%), 1 benign lymphadenopathy (3%), 1 acinic-cell carcinoma (3%), 1 adenoid cystic carcinoma

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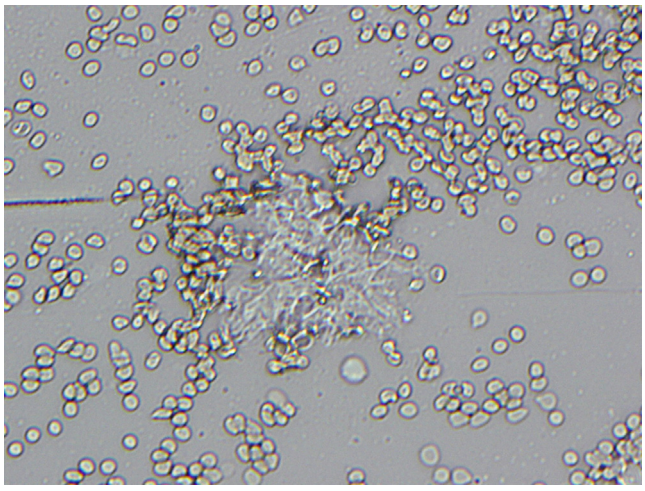

Figure 2 Rapid-on-site evaluation of a specimen adequacy, direct examination × 200. One cluster of parotid cells is observed on an adequate sample.

(3%), 1 low-grade mucoepidermoid carcinoma (3%), and 1 suspicion of basal-cell carcinoma (3%).

Relationship between cytology and histopathology

Final histological diagnoses were available for 24 cases. The cytological diagnosis was adequate in 20/24 cases: i.e., 12/12 pleomorphic adenomas, 4/5 Warthin's tumors, 2/2 lymphomas, 1/1 acinic-cell carcinomas, and 1/1 acinic-cell carcinoma. The cytological diagnosis was adequate to determine the presence of a benign tumor in 1/1 case (a cytological oncocytic tumor, which was a pleomorphic adenoma with oncocytic metaplasia), and for a malignant tumor in 2/2 cases (one low-grade mucoepidermoid carcinoma, which was a low-grade papillary cystadenocarcinoma, and one basal-cell carcinoma, which was an adenoid cystic carcinoma). One diagnosis was not adequate for a suspected Warthin's tumor, which was a low-grade mucoepidermoid carcinoma.

Discussion

Preoperative cytology helped to differentiate between benign and malignant lesions of the parotid gland: this enabled the extent of surgery to be planned and modified accordingly. Even though the use of US guidance has led to significant improvements in the quality of specimens, all published studies have reported variable percentages of inadequate samples. In this study, we suggest a method that can decrease or even remove this risk. It is usually the case, with inadequate samples, that the procedure has to be repeated, which results in causing greater patient discomfort, delayed diagnosis, and delayed surgery. With the method described herein, the occurrence of inadequate samples was zero. Consequently, patients did not require repeat punctures and the cytological diagnosis could be combined with data from the MRI (when available) to give the surgeon optimal visual information. Our results confirm the efficacy of US-guided fine-needle capillary cytology to diagnose parotid masses, and demonstrate that combining guided-US, without needing aspiration punches, and simultaneous on-site examination of slides by the radiologist, provided a useful technique for adequate sample collection. Furthermore, it was useful in cases where a negative puncture had been obtained in a different healthcare unit. Because it is known that parotid-mass punctures can often cause pain, the use of direct microscopy enabled a single puncture if the sample was adequate, which certainly benefited the patients.

We used a 25-gauge needle rather than aspiration. We believe that one of the most important factors of our technique was avoiding the vacuum effect caused in aspiration: our technique resulted in less blood contamination and higher quality material. The 25-gauge needle was hand-held and its upwards—downwards movement could be conducted using wrist movement instead of shoulder-joint movement, as is used in aspiration. This led to more sensitive and gentle handling of the lesion. Even though no aspiration was exerted, the passage of the needle through a capillary can easily capture cells [21,22].

The radiologist performed the microscopy after training with an experienced cytologist. This method has proved to be very successful. The optimal condition is when the cytologist is present at the same time as the radiologist, but this occurs rarely. In our institution, collaboration between radiologists and cytologists has led to training microscopy by direct examination, which has improved the results.

The definition of ''specimen adequacy'' is a matter of debate. We defined our samples as adequate if at least six parotid-cell clusters were found per slide on at least two slides, and was agreed to by the cytopathologist. Using this technique and this criterion, we obtained sufficient material to establish a diagnostic cytology in all cases. When compared to histological results, there was a very good correlation. The unique pitfall was represented by a low-grade mucoepidermoid carcinoma, which was diagnosed as a Warthin's tumor in the cytology and was caused by an inflammatory background with some oncocytic cells. In our study, three tumors were not strictly identified, but their malignant or non-malignant characteristic was diagnosed. Thus, the oncoming classification of the ''Milan System for reporting salivary-gland cytology'' would have helped to classify these cytologies.

Nevertheless, the main purpose of this study was to evaluate the impact of our fine-needle biopsy method and the utility of microscopy on the yield of adequate cytologic material. In this regard, our results show that our technique was highly advantageous for patients affected by parotid masses. The main advantage of improving the diagnosis of salivary-gland cytology is to provide the surgeon with maximum data to allow him/her either to perform conservative treatment or a parotidectomy according to the lesion, after discussion with the patient, including considering the risk of damaging the facial nerve [23]. FNC provided rapid-on-site assessment by the radiologist using microscopy. It was an effective time-saving method that could be used for needle punctures of the parotid or other salivary-gland masses, as well as for thyroid or head-and-neck lesions.

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Disclosure of interest

The authors declare that they have no competing interest.

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