Universitat de Girona

Facultat de Medicina

Role of Fine-Needle Aspiration Cytology in the Diagnosis of Parotid Gland Tumours: a Cross-Sectional Study

END OF TERM PROJECT

Oral and Maxillofacial Surgery Department

Hospital Universitari de Girona Doctor Josep Trueta

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I would like to thank Doctor Josep Trueta's Hospital Oral and Maxillofacial Surgery Department for their continued support. Specially my tutor Dr. Manel Gorina for teaching me with so much enthusiasm and for allowing me to discover my vocation and future way of life.

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Finally this project is dedicated to dear my family, my greatest support.

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1. ABBREVIATIONS

ASCP	American Society for Clinical Pathology
BACAF	Biopsia por Aspiración Con Aguja Fina
FNA	Fine- Needle Aspiration
FNAB	Fine- Needle Aspiration Biopsy
FNAC	Fine- Needle Aspiration Cytology
HDFSS	Histopathological Diagnosis From Surgical Specimen
HUDJT	Hospital Universitari Doctor Josep Trueta
NAB	Needle Aspiration Biopsy
OMSU	Oral and Maxillofacial Surgery Unit
PGCs	Parotid Gland Cancers
PGTs	Parotid Gland Tumours
SGTs	Salivary Gland Tumours

2. ABSTRACT

Background Fine- Needle Aspiration Cytology (FNAC) has been widely used as a diagnostic tool in clinical practice and is a well-established tool for investigation many head and neck conditions. Its application in Parotid Gland Tumours (PGTs) is however controversial: preoperative diagnose of parotid cancers remains an up-to-date issue in hospitals and still generates discrepancies among physicians. However, a false diagnosis for neoplasia can result a delay of treatment and progression of a disease.

In this sense, giving medical evidence of a reliable and accurate approach that provides adequate information on what concerns preoperative diagnosis is the endpoint of this project, because effective treatment for PGTs starts with an accurate preoperative diagnose.

Purpose The goal of the present work is to determine the accuracy of Fine-Needle Aspiration Cytology in the preoperative detection of Parotid Gland Tumours at the Hospital Doctor Josep Truetas Oral and Maxillofacial unit by calculating its sensitivity, specificity positive and negative predictive value and accuracy.

Design A prospective diagnostic test study with cross-sectional design carried out in a tertiary referral hospital in Girona, in a period time of three years.

Participants A total of 80 patients suspected of having parotid cancer who visit the Oral and Maxillofacial Service of Hospital Universitari Doctor Josep Trueta (HUDJT).

Keywords Accuracy, Diagnosis, Fine-Needle Aspiration Cytology, Parotid Cancer Parotid Gland Tumours, Parotid Neoplasm.

3. INTRODUCTION

3. 1. BACKGROUND

➢ 3. 1. 1. Parotid gland tumours: general approach

Parotid glands are exocrine organs responsible for the production and secretion of saliva. They form part of the group of salivary glands of our body. This group is formed by two groups of glands: On the one hand, major glands which are parotid, submandibular and sublingual gland and on the other hand, minor glands which are distributed throughout the mouth and oropharynx (1).

Parotids are the biggest salivary glands of the body with 2, 5 kg each one. We all have two glands situated on both sides of the head below the extern auditory conduct between jaws and sternocleidomastoid muscle. Zygomatic arch delimitates the superior limit and medially the masseter muscle (*See Figure 1*). Each gland has a major duct called *Stenon or Stensen Duct* which connects the parotid gland with the oral mucosa. *Stenon* duct opens in the rear of the mouth cavity near the second upper molar(2)(3).

Parotid glands are divided into two lobes (superficial and deep) separated by the facial nerve or VII cranial nerve (CN).

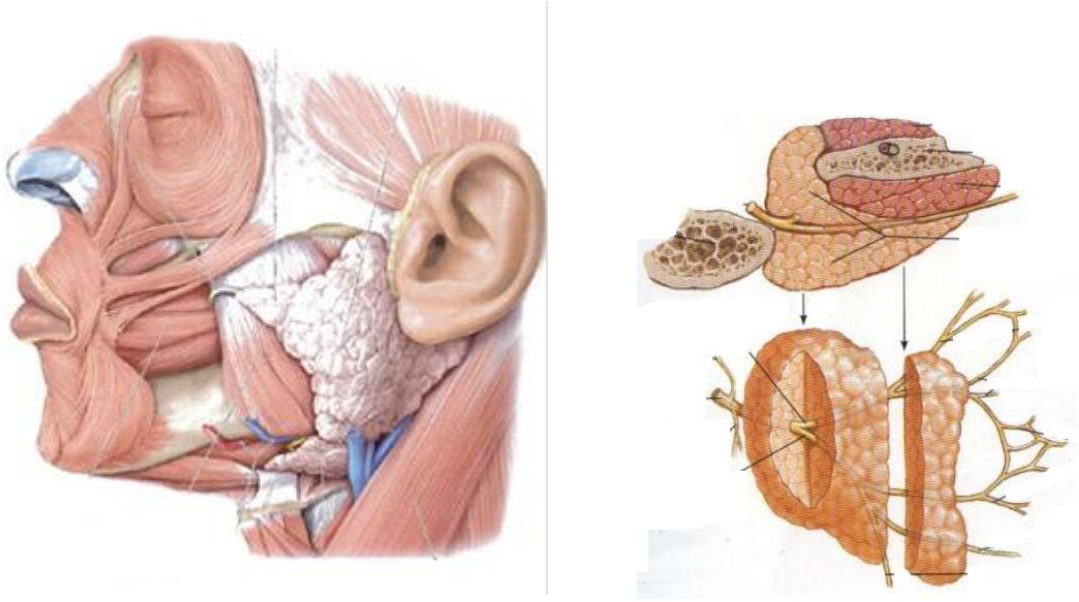

Figure 1: *Anatomy of the parotid gland: Left side, anatomic situation and relations of the gland. Right side, relation with facial nerve (VII CN)*
Author: *Left side* (3) *and right side* (4)

This is an important relation to have in mind, especially when parotid tumour surgery is done, because any excision that includes facial nerve can cause functional and aesthetic injuries as VII CN innervates the motor function of muscles of facial expression, neck and posterior belly of digastric. It is responsible of parasympathetic function of lacrimal, submandibular, sublingual and sweat glands, nasal and nasopharynx mucosa and auditory artery and its branches. It also handles the general sensitivity of the pinna and the external auditory conduct (*Ramsay-Hunt Zone*). And, finally, it is in charge of the anterior 2/3 taste sensitivity of the tongue(3)(4).

Histologically, parotids are composed of serous and mucous acini, the proportions of which determine the type of salivary secretion. Parotid is mainly serous and even is the biggest salivary gland of the body it only produces 30% of the total production of saliva. The importance of this saliva it is because of two reasons: The first one, is its high concentration of *ptyalin enzyme* which is an amylase whose function is the initiation of starch digestion; and the second one, is the secretion of immunoglobulin IgA which inactivates oral cavity antigens(5).

Unfortunately, this normal histology can suffer mutations and become tumours. Approximately 3 to 6 % of all head and neck tumours affect to the salivary glands. Concretely 65% of those are located on the parotid gland (2), especially on the superficial lobe. It should be noted that the majority (75%) of the parotid gland tumours are benign(6).

According to literature, annual incidence of SGTs oscillates between 0’4 and 6’5 cases/100.000 habitants^2(7). An estimated 650 deaths related to parotid gland tumours occur annually in the USA (6). Additionally, people with malignant parotid tumours have eight times more probabilities to suffer a second primary breast cancer. Also ovary (x5), respiratory (x2,5) and prostate (x3-4) cancers(8).

The histologic tumour distribution of the parotid gland is the following (See *Figure 2)*:

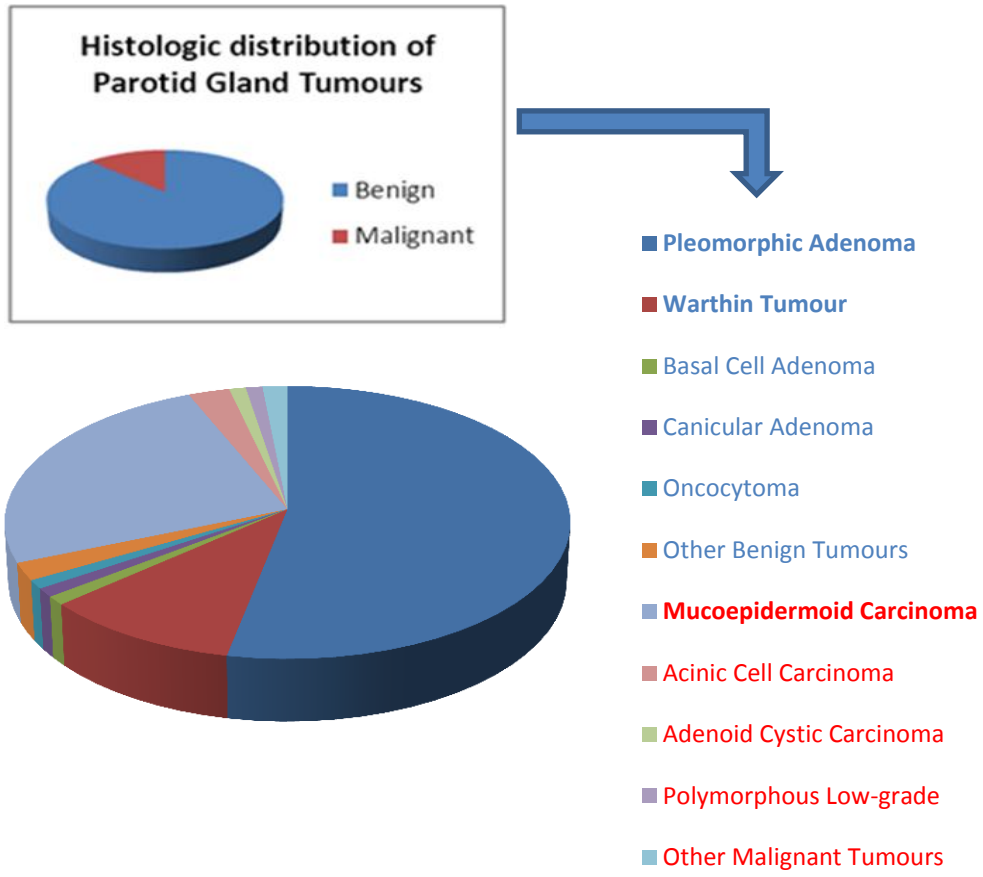

Figure 2: *Histologic distribution of parotid gland tumours *See detailed histologic types on* ***Annex I***
Author: *Marta Valls Gay, adaptation from text* (2)

As we can see on *Figure 2*, among all patients, the most common tumour type is pleomorphic adenoma, which is a benign tumour that accounts for about 50% of all tumours. Warthin tumour is second in frequency among benign and mucoepidermoid carcinoma is the most common malignant tumour. Among children mucoepidermoid carcinoma is the most frequent tumour. Another peculiarity among tumours in childhood is that mesenchymal tumours are more common than in adults (7).

Females are most frequently affected but there is some gender variation according to the tumour type. For instance, pleomorphic adenoma is more common on men and mucoepidermoid carcinoma affects more women.

The average ages of patients with benign and malignant tumours are 46 and 47 years, respectively, and the peak incidence of most specific types is in the sixth and seventh decades. However, the highest incidence of pleomorphic adenomas and mucoepidermoid carcinomas is in the third and fourth decades (1).

Aetiology of parotid cancer is not known. Some risk factors have been studied such as cigarette smoking, genetic predisposition, hormone influence, viral infections (VEB, CMV), rubber manufacturing, plumbing, some types of woodworking, asbestos mining, kerosene and exposure to cement and silica dust, kerosene and nickel compounds. However, there are only two well-established risk factors: One the one hand, ionizing radiation. Radiation to the head and neck increases the risk of malignancy of salivary glands with a 15- to 20-year latency (9). This association was established using the survivors of atomic bomb explosions in Hiroshima and Nagasaki. Long-term follow-up studies showed an increased relative risk of both benign and malignant parotid gland tumours. Related to this risk factor it is important to notice that therapeutic radiation and routine dental radiographs have been linked with a significantly increased risk of developing PGCs. On the other hand, cigarette is related to Warthin tumour. In fact, this tumour occurs eight times more often in smokers than in non-smokers(1).

The most common clinical presentation of PGTs is the appearance of a lump. As main parts of parotid tumours are located on superficial lobe and as most of them are benign, the most typical place is parotid gland queue, in front of the earlobe as a moveable and elastic mass. Most of them are asymptomatic, so they are a "*casual finding*". Malignant tumours are most frequently located on the profound lobe. They appear as a feeling of fullness in the retromandibular portion of the gland and tend to cause peripheral facial nerve paralysis(2)(See *Figure 3*).

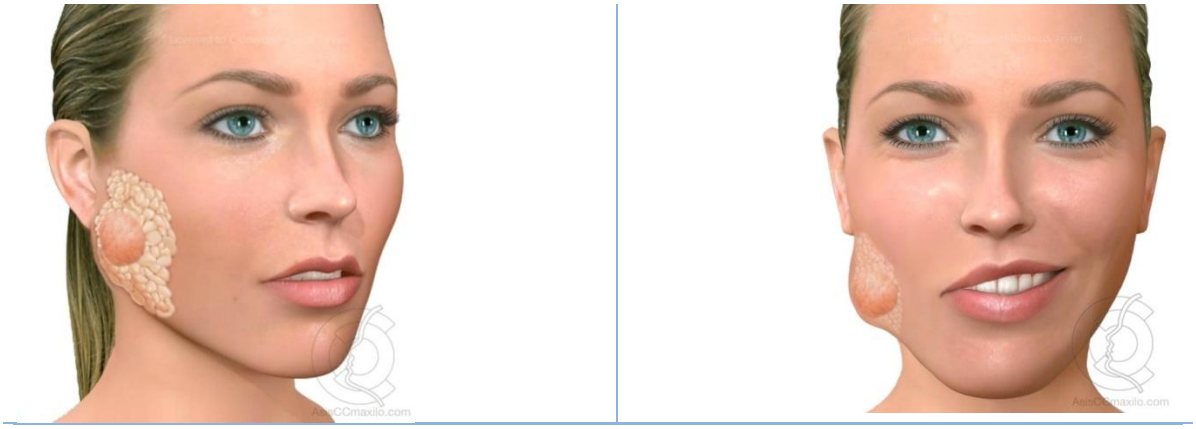

Figure 3. *On the left side we can see typical clinical presentation of benign parotid tumours and on the right side typical malignant presentation. *See* ***Annex II*** *Rankow criteria*
Author: *Doctor Calderón Polanco* (10)

'*Red flag'* features suggesting the possibility of malignancy include: facial nerve weakness or paraesthesia, rapid increase in the size of the lump, lymphadenopathy, ulceration or induration of the skin, intermittent paint, clinical history of previous skin cancer, radiation to the head and neck or Sjögren syndrome (9).

The diagnosis of PGTs is mainly clinical by using patients clinical history and signs and symptoms. There are some clinical manifestations summarized in the *Rankow Criteria* (See *Annex II*) that may be useful for guiding the physician and that are independent of the tumour type.

Although *Protocolos de la Sociedad Española de Cirurgía Oral y Maxilofacial* (6) and *Jatin Shah* (2) express that "*main part of patients that have a benign clinical picture rarely need an histological diagnosis before surgery is practised*", in our country Spain independently of clinical diagnosis compulsory FNAC is done after clinical examination.

FNAC is a diagnostic technique that uses a thin needle to take a small sample of tissue from an organ or tumour cells through a syringe attached to the needle. When the sample is removed, a pathologist examines it to find the final diagnosis. It can be performed by palpation or ultrasound or Computerized Tomography (CT), according to the site of injury and the characteristics of the tumour. In parotid gland concretely usual procedure is by palpation or ultrasound guiding. FNAC can provide rapid, non-surgical diagnose and it can be performed at the time of initial consultation. Complications are rare but can occur such as vasovagal syncope or local hematoma (haemorrhage) in the zone of the lesion we are studying (11).

Detailed step procedure of Fine-Needle Aspiration Cytology will be further explained on *Section 8.6.* and *Annex III.*

Even though it is controversial, and all points of view of this technique will be commented forward on *Section 3.1.2.*, FNAC in experimented hands provides information of two key decisions in patient management: First, differentiates between neoplastic and nonneoplastic lesions. Neoplastic lesions usually will be managed surgically, whereas nonneoplastic lesions conservatively. Second, given a neoplastic lesion, FNAC determines whether the lesion is malignant o benign, which determines the extent of surgery and, in particular, whether the facial nerve can be spared during surgery (6)(12).

Incisional (tissue) biopsy of the parotid gland, which is sometimes practised in minor glands diagnose carries significant risks, including fistula formation, facial nerve damage, sampling error, violation of tissue planes and, occasionally, poor placement of an incision makes subsequent surgery more difficult. The main objection of this technique is the possibility of tumour implantation by the possibility to spread tumour cells. This last point has to be highlighted because studies have proven the safety of FNAC on the seeding of neoplastic cells, which makes FNAC a safe procedure for the diagnosis of parotid gland tumours (13).

Depending on each case, diagnosis extension will be done using Computerized Tomography (CT) and /or Magnetic Resonance Imaging (MRI). Ultrasonography or Gamma-scan may be useful for some histological types (Warthin tumour). Plain radiography and sialography are rarely useful in PGTs diagnosis(2)(6).

Correlation of the clinical impression, cytological diagnosis and radiographic imaging studies can establish the tumour staging (*See Annex I*) and then guide along different treatment pathways.

Essential goal of parotid cancer treatment is the control of the illness and, whenever is possible, conservation of gland function in order to decrease morbidity (6). This last point is especially important in malignant tumours when resection of the facial nerve becomes a fundamental decision.

Radical surgical excision (parotidectomy) is the cornerstone treatment of PGTs (14). It should be pointed out that only after surgery we will have definitely diagnosis by

doing the histopathological diagnose of the surgical specimen, which is considered the *Gold Standard* for the diagnosis of parotid gland tumours.

Type of surgery practised will depend on two factors: First one, tumour characteristics (size, primary localization and histological stage, *detailed on Annex I*) and second one, patients profile (presence of systemic pathology, age and social conditions)(2)(6). Together with surgery other complementary treatments can help in determined situations in order to control loco regional illness. Among these treatments radiotherapy complementary to surgery is the election.

Treatment options are the following (See *Table 1*):

<table>
<tr><th colspan="2">OPTIONS OF TREATMENT</th><th>CLINICAL CONDITION</th></tr>
<tr><td rowspan="3">A</td><td rowspan="3">Superficial parotidectomy
or
Total conservative parotidectomy</td><td>Anatomic stages 1 , 2</td></tr>
<tr><td>Low malignancy grade</td></tr>
<tr><td>No facial nerve affectation</td></tr>
<tr><td rowspan="4">B</td><td rowspan="4">Radical parotidectomy
or
Extended parotidectomy</td><td>Anatomic stages 3 , 4</td></tr>
<tr><td>High malignancy grade</td></tr>
<tr><td>Facial nerve affectation</td></tr>
<tr><td>Certain tumour recurrence</td></tr>
<tr><td colspan="3">• Radiotherapy can be used post operatory to advanced stages in order to increase locoregional illness control and survival.
• It is also convenient to think in the possibility to use nerve graft in order to decrease morbidity. If this last option cannot be possible early facial rehabilitation should be started.
• If positive lymph node or advanced tumour stage lymphadenectomy is indicated.
• Sometimes when insufficient surgical excision is done, jaw osteotomy should be practiced.</td></tr>
<tr><td rowspan="2">C</td><td rowspan="2">Alone radiotherapy</td><td>Unresectable tumours</td></tr>
<tr><td>Certain tumour recurrence</td></tr>
</table>

Table 1. *PGT treatment options* (6)

Regarding to prognosis there is a strong correlation with clinical stage, emphasizing the importance of early diagnosis (14). For example, 3 and 4 stages have 30% of survivors after 5 years unlike 1 and 2 stages which have a 90-95% survival (6). Hence, we go back to importance of an *accurate diagnosis* because an early and correct diagnosis improves prognosis and chances of cure.

➢ 3. 1. 2. FNAC: Is it useful?

Fine- needle aspiration cytology (also called FNAB, FNA, NAB or BACAF) has been widely used as a diagnostic tool in clinical practice for 150 years and since 1980's had been used and it is a well-established tool for investigation many head and neck conditions (15)(16). Its application in parotid tumours is however controversial (12)(15)(16)(17)(18)(19).

Widespread acceptance regarding the usefulness of FNAC exists. There are institutions where it is performed as a mandatory procedure before surgery and at the other extreme some authorities used it occasionally to gather additional supporting evidence when deciding for conservative treatment.

On the one side, the opponents questioned the clinical usefulness of FNAC because it is an operor-dependent technique with low sensitivity and high false negative rates (12). As eighty per cent of the parotid tumours are benign their opinion is that knowing the FNAC results rarely affect the type of surgery performed because what determines the extend of surgery is the position of the tumour within the parotid gland, not the tissue diagnosis (20).

There are two figures to highlight in the opponents side: The first one is *Batsakis et al* (21) and the second one is *Olsen et al* (22). *Batsakis* has the conviction that preoperative FNAC had little influence on clinical management and suggested that cytological assessment should be limited to certain patient groups such as those unfit to surgery, or children, in whom the risk of neoplasia is low. *Olsen* focused on the high false negative rates previously commented and, as well as *Batsakis*, they drawn the attention to the rare but potentially serious effects of prior needle sampling on subsequent histological assessment of salivary gland lesions such as metaplasia, infarction, necrosis, fibrosis, granulation or haemorrhage (23)(24).

On the other side, the proponents of FNAC for parotid tumours feel that it has multiple advantages. It is a non-expensive, simple, quick and safe technique (15)(25). It provides preoperative information about the malignant nature of parotid lesions which can be helpful in assessing and establishing a policy toward neck lymph nodes, achieving wide tumour-free excision margins, preventing delay and informing the patient more appropriately on the treatment plan and on the possible risk of facial nerve injury. Thus in case of a benign tumour, surgery can be postponed or the patient can be followed if general health or other medical conditions pose a major surgical risk (18)(20). Finally, according to proponents (26), thanks to FNAC, unnecessary surgery can be avoided in approximately one third of cases.

3. 2. JUSTIFICATION

The clinical usefulness of fine-needle aspiration cytology for the diagnosis of parotid gland lesions is controversial (12)(15)(16)(17)(18)(19). In fact, a Meta-Analysis published in 2011 by the American Society for Clinical Pathology (ASCP)(12) concluded that "*It is not possible to provide a general guideline regarding the clinical usefulness of FNAC for parotid gland lesions*" they noticed that all the studies that investigate the accuracy of FNAC that were previously published showed a large variability in the results (sensitivity rates between 33% and 100% and specificity rates between 67% and 100%).For this reason, ASCP finally suggested in this meta-analysis that new studies should be done with better quality of reporting and better designs to remove or assess the impact of bias in order to give a general guideline of use.

Our study tries to solve this problem. It gives new perspective as it will be different from the others published because of its prospection, its blinding and detailed steps procedure in a trained clinical setting. We analyse the literature and tried to minimize the bias: On the one hand, the largest number of studies published in the 2011 ASCP Meta-Analysis where conducted in the United States and only two eligible where published in Spain (27)(28)both were retrospective and in fact, among 64 of the studies analysed only two were prospective studies. On the other hand, we tried to minimize the errors by reporting the experience of the pathologist and specifying who is obtaining the sample. Finally, as only two studies were blinded, we decided blind the steps in order to reduce bias.

To conclude, preoperative management of PGCs remains an up-to-date issue in hospitals and still generates discrepancies among physicians. However, a wrong diagnosis for neoplasia can result a delay of treatment and progression of a disease, which can cause not only esthetical and functional problems such as facial nerve paralysis but also second primary tumour, and even life expectancy or death of the affected people. In this sense, giving medical evidence of a reliable and accurate approach that provides adequate information on what concerns preoperative diagnosis is the endpoint of this project, because effective treatment for PGTs starts with accurate preoperative diagnose.

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5. QUESTION

Is Fine-Needle Aspiration Cytology a reliable approach in the preoperative assessing of Parotid Gland Tumours when compared to histological analysis (*Gold Standard*)?

6. HYPOTHESIS

Preoperative FNAC is a reliable technique for the detection of parotid tumours compared to postoperative histopathological examination (*Gold Standard*).

7. OBJECTIVE

The main target of this work is to evaluate the sensibility and specificity, positive and negative predictive value and accuracy of FNAC in the detection of parotid tumours.

8. MATERIAL AND METHODS

8. 1. STUDY DESIGN

This protocol consists in a prospective cross-sectional study for a diagnostic technique, which will be carried out by the Oral and Maxillofacial Surgery Unit in the tertiary referral Hospital Doctor Josep Trueta of Girona during three years.

8. 2. POPULATION

To know which patients are going to participate in this cross-sectional study we have to define our population of interest. This group will be people suspected of having parotid cancer who visit the Oral and Maxillofacial service of Hospital Universitari Doctor Josep Trueta (HUDJT).

8. 2. 1. Inclusion criteria

- Patients (men and women) visited in HUDJT who present a parotid node suggestive of neoplasia.
- Patients must have preoperative FNAC of the parotid gland.
- Patients must have a postoperative biopsy of the surgical specimen.

- **8. 2. 2. Exclusion criteria** (29)
  - Patients with too few cells in the FNAC ("*unsatisfactory sample*") or FNAC with non-conclusive diagnosis ("*uncertain diagnosis*").
  - Patients who have more than one FNAC preoperatively, because prior needle sampling can change normal structure of the cells (inflammation, haemorrhage and scarred areas).
  - Patients pregnant, because pregnancy sometimes can increase cell size in *Papanicolau* smears.
  - Patients who have coagulation problems, because haemorrhage cause false positive and false negative results in the FNAC.
  - Patients with previous FNAC done in other sanitary centre because we cannot control procedure bias such as pathologist and maxillofacial specialist experience.
  - Patients who received radiation therapy to the head and neck region because radiotherapy induces cellular changes and consequently diagnostic pitfalls due to FNAC.
  - Patients with ultrasound guided FNAC, because ultrasound is not the habitual procedure in our service.
  - Patients who have previous homolateral parotidectomy to actual parotid tumour situation, because surgery can make inflammatory tissue changes which may obscure cellular details.
  - Inability to understand and sign an informed consent form.

8. 3. SAMPLE SELECTION

The sample will be consecutive so it won't be probabilistic. This means that every patient who attend to our department or referred to us and which meet inclusion criteria and not exclusion will be enrolled in this cross-sectional study.

The sample recruitment will take place in HUDJT throughout 2 years and every patient will be asked to participate in this study. If the subject agrees, the principal investigator will give him/her an informed consent for participating in this project (*Annex IV*) and for doing the surgery as usual.

➢ 8. 3. 1 SAMPLE SIZE

Sample size has been calculated using GRANMO application. We considered 99% sensitivity on the histopathological examination group and 90% sensitivity on the FNAC group.

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 80 subjects are necessary in first group (FNAC) and 80 in the second (histopathological examination) to find as statistically significant a proportion difference, expected to be of 0.99 in group 1 and 0.9 in group 2. It has been anticipated a drop-out rate of 0%. ARCSINUS approximation has been used.

As in the Oral and Maxillofacial service of our centre, approximately 45 parotid gland tumours are diagnosed in a year and each of them have a FNAC diagnosis and a histopathologic diagnose from surgical specimen we consider the field work will be two years duration.

8. 4. VARIABLES

VARIABLES		***Type of data***	***Measure instrument***	***Categories or values***
Main variables	**FNAC**	Dichotomous qualitative	Pathologist diagnosis	Positive / negative
	HDFSS	Dichotomous qualitative	Pathologist diagnosis	Malignant / Benign
Covariables	**Age**	Discrete quantitative	Clinical history, anamnesis	Number of years
	Gender	Dichotomous nominal qualitative	Clinical history, anamnesis	Male or Female
	Dimension of the tumour	Quantitative continuous	Pathologist diagnosis of the surgical specimen	Millimetres^3 (A X B X C)

➢ 8. 4. 1. MAIN VARIABLES

a). FNAC results: cytological diagnosis of a malignant parotid tumour will be classified as a positive result, whereas diagnosis of a benign tumour will be classified as a negative result. Aspirates with too few cells that are scored as "*uncertain diagnosis*" or "*unsatisfactory sample*" will be left out of the analysis.

b). Histopathological diagnosis from surgical specimen results: surgical specimen will be classified as malignant or benign according to pathologist criteria based on her or his knowledge as they will be experts in parotid tumours examination.

➢ 8. 4. 2. COVARIABLES

These variables will be collected in order to avoid confusion as they can act as confusion factors and alter the interpretation of the results.

a) **Age:** in years
b) **Gender:** male or female
c) **Dimension of the tumour:** will be expressed in millimetres^3, result of measuring the width (mm) x height (mm) x length (mm)

8. 5. DIAGNOSTIC TESTS

The tests that are going to be compared are represented by the technique used for preoperative diagnosis of parotid tumours (FNAC) and by the postoperative histopathological diagnosis obtained from the surgical specimen. The procedure of the following techniques will be explained on the data collection (*See 8.6*.).

8. 6. DATA COLLECTION AND STUDY CIRCUIT

In our project two departments will work together for the data collection following a specific circuit, explained below.

On the one hand, a surgeon from the Oral and Maxillofacial Surgery department, previously informed and coordinated for the study as well as trained and experienced for doing FNAC

and parotid surgery; and on the other hand, two members of the Pathology Department, one previously formed on FNAC and the other specialized in parotid biopsy interpretation.

Information will be collected on Case Report Form (CRF: see *Annex V).* In order to preserve confidentiality, patients will be identified in the CRF with numbers given consecutively as they attend to our department.

a). Maxillofacial specialist: according to the clinical findings and patients history, will detect a parotid suggestive of neoplasia (See *Rankow Criteria Annex II*). The first step to do for the data collection will be the acquisition of the signed informed consent (*Annex IV*) after the patient has read a general informative document (*Annex VI*).

Once the patient has read and understood all the procedures, the FNAC will be done using the following material (See *Figure 4*):

1). Short needle (20 G calibre and 1, 5 cm length)

2). Syringe with straight piston (20 cc)

3). *Cook Medical* cytology gun

Figure 4: *FNAC material*
Author: *Marta Valls Gay*

Physician will try to relax patient by explaining the innocuousness and few discomforts of the technique. The patient will be in supine decubitus with 30º inclination of the head which will be also contralateral rotated to the area that needs to be investigated.

After antiseptic cleaning of the hands (using *Sterillium®*e.g.) and colocation of sterile gloves, the maxillofacial specialist will look for the suspicious node and disinfect the skin with sterile gauze impregnated with iodine or alcohol 96%.

Then, with the non-dominant hand will stretch the skin of the zone in order to reduce pain while fixing the node with the fingers **(A)**. Straightaway, with the dominant hand the clinic will do the puncture itself. For achieving this, the needle will be introduced inside the lesion firmly and vertically. The next step will be a soft aspirating of the material and with this negative pressure slow inside out rhythmic and uniform movements in the different directions of the lesion will be done until needle is full of material **(B)**. When this occurs, piston has to be slowly released to its rest position in order to eliminate negative pressure and after this, needle can be carefully removed **(C)**.

*Letters of the text correspond to *Figure 5* where this procedure is detailed

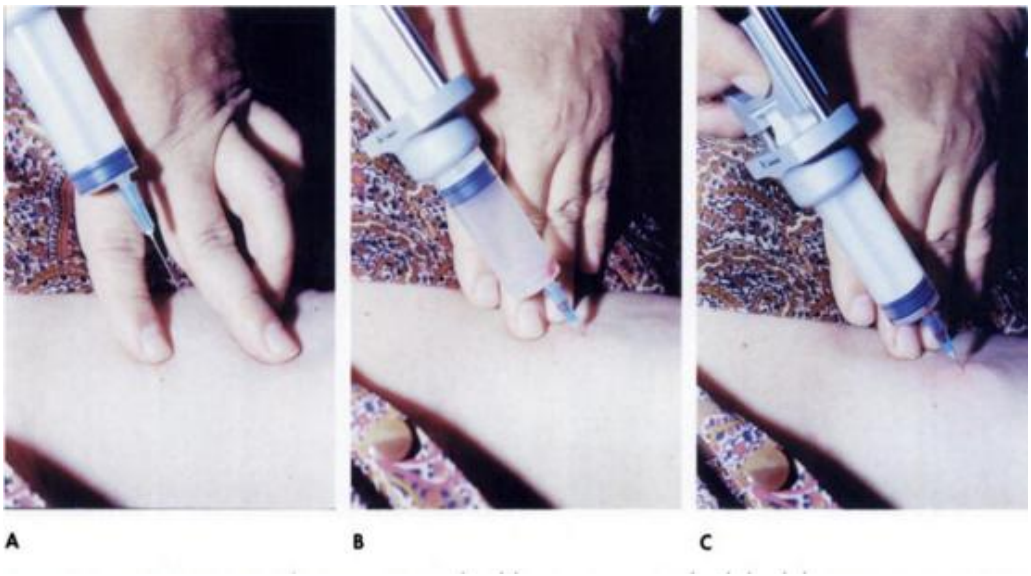


Figure 5: *FNAC puncture procedure.* ***A).****fixation of the node with fingers with the non-dominant hand while needle is firmly introduced.* ***B).*** *Aspiration of the material while doing rhythmic movements* ***(C)*** *Needle full of material. Reposition of negative pressure and removing needle.*
Author:(11)

As needle is removed, immediately the physician will have to make pressure to the punctured zone with sterile gauze in order to stop the bleeding and after a few seconds we will proceed to put simple dressing.

The nurse of the OMSU will have to stick correctly the patient information and send the sample (syringe + gun) to Pathology Department the same day FNAC is practiced.

Maxillofacial specialist will have to note on the Case Report Form the clinical diagnosis, which will be blinded for the next step.

b). Pathologists: will receive the sample. The analysis will be blind in order to reduce verification bias.

Pathologist will examine the FNAC the same day the sample is received. The procedure, illustrated in *Annex III*, consist in the following steps:

The initial smears will be stained by quick stain (stains, which needs approximately one minute to perform) such as *Diff Quick (DQ)* stain or *May Grumwald Giemsa.* This type of stain is done on slides with material after air-drying and this is why they are also called air-dried stains. This type of stain is good for cytoplasm, and background material staining.

The other set of slides are fixed in basic ethanol-based solution (96% ethanol) for different type of stain, the *Papanicolau stain*. This stain is more superior for demonstrating the nuclear details, which are the most important and specific in making the diagnosis of malignancy.

In addition, to the previous smears, the rest of the material will be flushed in an ethanol solution after the material is centrifuged and a small mini biopsy is created from the concentrated cellular material at the bottom of the tube. This is known as *cell block*. The slides of this cell block will be stained with *Haematoxylin and Eosin stain* and will be collected for further studies.

Smears will be studied on the microscope and pathologist will have to write a diagnosis that will be collected on the clinical history.

c).Maxillofacial specialist: One week after FNAC will visit the patients and explain the FNAC results. Results of the FNAC will be recorded on the CRF.

Surgery will be done according to the results of the puncture in such a way that positive FNAC for malignancy will be performed maximum three weeks after the puncture and negative FNAC for malignancy will be performed maximum four months after the puncture.

When intervention is done, the operation room nurse will have to stick correctly the patient information and send the parotid piece to Pathology Department the same day the surgery is practiced.

d). Pathologist: Once the surgery is done, the surgical specimen will be examined. Again, the procedure will be blind. Not knowing the clinical or FNAC diagnosis. The procedure will consist on the following steps:

Firstly, the status of surgical margin will be assessed by applying ink to all the surfaces of the specimen. Afterwards, the surgical specimen will be cut in thick slices. Following that, the pathologist will assess the macroscopic margins of the sliced specimen using an adapted ruler and a macroscopic description will be done (*See Figure 6).*

Figure 6 : *On the left side, inked surgical specimen being cut and macroscopic cuts and on the right side, cuts after macroscopic description ready to be processed.*
Author: *Marta Valls Gay*

The material will be fixed in neutral buffered formaldehyde and processed in paraffin blocks according to standard procedure. Section 4 µm thick will be cut and stained with *Haematoxylin and Eosin stain*. These samples will be analysed through the microscope.

It is important to remember again that nowadays, the pathologist evaluation of the excised specimen is considered to be the *Gold Standard* for parotid gland tumours diagnose and that is why this step will be very important in our project.

At the end of the procedure the pathologist will have to note the diagnosis on the clinical history of the patient.

e). Maxillofacial specialists: will meet the patient two weeks after surgery in order to control the surgical complications and to see the results of the biopsy of surgical specimen. The results will be again noted on the Case Report Form.

9. STATISTICAL ANALYSIS

Data will be introduced in the database (*Microsoft Access 2014*) and statistical calculations will be performed using the Statistical Package for the Social Sciences programme (*IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.*)

9.1. UNIVARIATE ANALYSIS

The univariate or descriptive analysis of the qualitative variables will be done using proportions while the univariate analysis of the quantitative variables will be performed using mean ± standard deviation if we can assume a normal distribution and if not, using the median.

9.2. BIVARIATE ANALYSIS

In order to describe the relationship between the two techniques, contingency tables will be performed. These will link the results of the FNAC with the results of histopathological diagnosis from surgical specimen. Using that information, sensitivity, specificity, false positives, false negatives and accuracy will be calculated (See *Annex VII*).

9.3. MULTIVARIATE ANALYSIS

For attaining the third endpoint, a multivariate analysis will be performed to adjust confusion and analyse the relationship between our primary endpoint with other covariates in our study. For this reason, in order to avoid potential confounders and to give more external validity to our study a logistic regression models will be performed to adjust for *age, gender and tumour size*.

10. ETHICAL ASPECTS

This study will be conducted according to the ethical principles established by World Medical Association in the Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects. The research protocol must be presented and submitted for consideration, guidance and approval by the *Clinical Research Ethical Committee* (CEIC, "*Comitè Ètic d'Investigació Clínica*") at Hospital Universitari Doctor Josep Trueta before the study begins, and at the end of the study, the final report must also be submitted to the CEIC.

According to "*Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal*", personal and clinical information of participants will be confidential and only used for purpose of research. Moreover, all data will be analysed anonymously.

All participants will be personally informed by researchers and an information document about the study will be given to them (*Annex VI*). Participants will have to sign voluntarily the informed consent (*Annex IV*) before being included in the study after receiving the appropriate information about procedures, according to "*Ley 41/2002 Básica reguladora de la automomía del paciente y de derechos y obligaciones en materia de información y documentación clínica"*.

11. LIMITATIONS OF THE STUDY

Some limitations that have to be known in the present work are:

- As FNAC will be performed by the same surgeon a procedure bias could occur. We will try to minimize it by the personal experience and training but it is important to have it on mind.

- Pathologist procedures (FNAC and surgical specimen examination) are operor-dependent techniques that require trained pathologist. Although the enrolled specialist will be trained so procedure bias will be minimized, a pathologist learning curve will be observed, as it happens with all technician dependent methods.

- As having ultrasound guided FNAC is an exclusion criteria we could have a selection bias because we probably lose all those people who have small or not well defined tumours which are candidates to do ultrasound guiding.

- As the use of the informed consent could be a selection bias response rate will be calculated in order to consider this bias.

12. WORK PLAN

The research team will carry out all the tasks of coordination, interpretation and dissemination of the results. The sequence of activities developed and entire research team is detailed below. Visual chronogram of the work plan is represented on the time schedule of *Section 13.*

Researchers: Manel Gorina (MG) and Marta Valls (MV)

Collaborators:

- Pathologist 1 specialized in parotid gland cytology interpretation (P1)
- Pathologist 2 specialized in parotid histopathological interpretation (P2).
- Nursing staff (NS)
- Statistician (ST)
- External collaborator, monitor function (EC)

The cross-sectional study has been designed in eight stages:

a) **<u>Stage 0:</u> Study design (3 months). This step is completed**
   - Researchers MV and MG.
   - Bibliographic research and protocol elaboration.

b) **<u>Stage 1:</u> ethical evaluation of the protocol (1 month)**
   - Protocol will be presented to the Ethics Committee of Clinical Research of IAS

c) **<u>Stage 2:</u> coordination phase (2 months)**
   - All investigators and collaborators will meet to coordinate the beginning of the study. All of them will be informed about the work plan and schedule and methods of data collection.
   - Pathologist enrolled in our project will be experts in parotid gland this means they have considerable experience in this type of tumour. P1 will have knowledge in parotid gland cytology interpretation and P2 in parotid histopathological interpretation. They will always be the same physicians in order to control procedure bias.

d) **<u>Stage 3:</u> Field research (24 months)**
   - **Pick patients:** Researchers MG and MV will include in the study every patient that come to our office and that meet inclusion and no exclusion criteria (consecutive sampling).
   - **FNAC** :
     - Researchers MG and MV will practice the puncture.
     - Pathologist P1 will examine the cytology
   - **Intervention:** researchers MG and MV will perform the surgery.
   - **Histopathological diagnosis from surgical specimen:** Pathologist P2 will examine the surgical specimen.

e) **<u>Stage 4:</u> Data collection (24 months)**
   - MG, MV, P1, P2, NS, EC.
   - Simultaneously with field research development, data will be registered in the Case Report Form. An analysis of data will be performed regularly by and external collaborator (EC) to control its evolution and verify that the protocol is being followed.

f) **<u>Stage 5:</u> Revision of all the data (1 month)**
   - All investigators.
   - A second meeting will be done to review all the data.

g) **<u>Stage6:</u> Statistical analysis (2 months)**

- ST.
- Data will be analysed using the appropriate statistical test.

h) <u>Stage 7:</u> Interpretation of the results (2 months)

- Researchers MG and MV.
- A results interpretation will be performed and the corresponding articles will be written.

i) <u>Stage 8:</u> Publication of the results (1 month)

- Researchers MG and MV.
- Write and edit the articles to publish them.

13. TIME SCHEDULE

CHRONOGRAM													
	Nov 2015-Jan 2016	**Feb-Apr 2016**	**May-July 2016**	**Aug-Oct 2016**	**Nov 2016-Jan 2017**	**Feb-Apr 2017**	**May-July 2017**	**Aug-Oct 2017**	**Nov 2017-Jan 2018**	**Feb-Apr 2018**	**May-July 2018**	**Aug-Oct 2018**	**Nov 2018-Jan 2019**
Stage 0: Study design													
Stage 1: Ethical evaluation													
Stage 2: Coordination phase													
Stage 3: Field research													
Stage 4: Data collection													
Stage 5: Revision of all the data													
Stage 6: Statistical analysis													
Stage 7: Interpretation of the results													
Stage 8: Publication of the results													

14. FEASIBILITY

In order to put this project into action we will form suitable medical team and a multiprofessional team. The main investigator will be the surgeon who has considerable experience in the surgical treatment of parotid gland tumours and in the practice of FNAC. The nurse who will be involved will be the habitual nurse that has been working with our department and who is used to FNAC procedure.

As our team does not habitually have specialized professionals in statistics we will hire an eternal statistician to do the statistical analysis.

Visits, surgical interventions and pathologist exams are usual performed at the Universitary Hospital of Girona Doctor Josep Trueta. We have a consultation room available 3 days a week as well as an operation room available one day per week and a pathologist department available 40 hours a week. The nurse, surgeon and pathologist are hired by the National Health System as well.

At our hospital we have around 45 patients per year diagnosed of parotid tumour, so in 2 years we can get the sample size.

15. BUDGET

In the feasibility section (*See Section 14)* is described the means available. As we have said in work plan (*See Section 12*) we will hire a statistician to do statistical analysis. Also, in order to control the evolution and verify the protocol is being followed we will hire an external collaborator (monitor).

The surgery as well as pathologist procedures (cytology and histopathology diagnose) and also the material needed to do FNAC are included in the National Health Service provisions and are common procedures which does not mean additional health cost.

EXPENSES			COSTS (€)
1. Personal expenses (Staff)			
2. Executive expenses			
• **Services procurement**			
- Monitor (EC)	1h/month x 24 months	20 € / hour	480 €
- Statistician (ST)	20h /week x 2 months	25€ / hour	4.000 €
3. Publication and dissemination expenses			
• **Scientific publications**			1.500 €
TOTAL			**5.980 €**

16. ANNEXES

16. 1. ANNEX I: Histology and staging

➢ 16. 1. 1. PAROTID TUMOURS HISTOLOGY

The histological classification recommended is a modification of the World Health Organization (WHO) classification of salivary gland tumours:

- **Epithelial tumours:**

This group represent the majority of parotid tumours, and in fact, the main type of SGTs . We can divide them on two types of tumours:

Benign epithelial tumours	Malignant epithelial tumours
- Plemomorphic adenoma *- Basal cell adenoma* *- Canicular adenoma* *- Warthin tumour* *- Oncocytoma* *- Sebaceous adenoma* *- Lymphadenoma* *- Ductal papillomas* *- Cystadenoma* *- Myoepithelioma*	*- Mucoepidermoid carcinoma* *- Adenoid cystic carcinoma* *- Acinic cell carcinoma* *- Polymorphous low-grade adenocarcinoma* *- Epithelial- myoepithelial carcinoma* *- (Hyalinizing) Clear cell carcinoma* *- Basal cell adenocarcinoma* *- Sebaceous adenocarcinoma* *- Sebaceous lymphadenocarcinoma* *- Cystadenocarcinoma* *- Low- grade cribiform cystadenocarcinoma* *-Mucinous adenocarcinoma* *- Oncocytic carcinoma* *- Salivary duct carcinoma* *- Adenocarcinoma, not otherwise specified* *-Myoepithelial carcinoma (malignant myoepithelioma)* *- Carcinoma ex pleomorphic adenoma* *- Carcinosarcoma (true malignant mixed tumour)* *- Metastasizing plemorfic adenoma* *- Squamous cell carcinoma, primary* *- Undefferentiated carcinoma, large cell type* *- Lymphoepitelial carcinoma* *-Sialoblastoma* *-Mammary analoge secretory carcinoma* *-High-grade neuroendocrine carcinoma*

- **Non epithelial tumours:**

Non epithelial neoplasms are rare, representing about 2-5 % of salivary gland tumours. They include, among others, *haemangioma, lymphangioma, schwannoma, neurofibroma, lipoma, sarcoma, lymphoma, and metastatic lesions* (which develop preferentially in the parotid glands, and are most often of squamous cell origin).

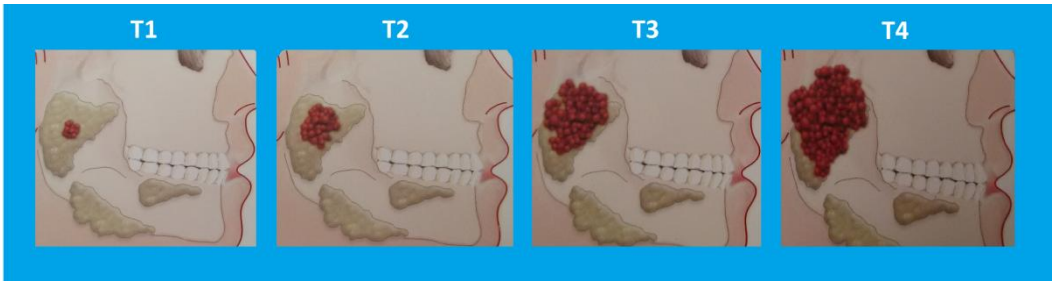
➢ 16. 1. 2. PAROTID TUMORS STAGING

The parotid tumours classification is based on TNM defined by UICC and AJCC (American Joint Committee on Cancer): (1) Primary Tumour; (2) Regional Lymph Node; (3) Distant Metastasis

- **Primary Tumour (T)**

Tx		Primary tumour cannot be assessed
T0		No evidence of primary tumour
T1		Tumour ≤ 2 cm in the greatest dimension without extraparenquimatous extension*
T2		Tumour ≥2 cm but no more than 4 cm in the greatest dimension without extraparenquimatous extension *
T3		Tumour > 4cm and /or extraparenquimatous extension*
T4	**T4 a**	Local illness moderately advanced Tumour that invades skin, jaws, ear canal and /or facial nerve
	T4 b	Local illness very advanced Tumour invades cranial base and /or pterygoid apophasis and /or surrounds carotid artery

**Extraparenquimatous extension implies clinical evidence or macroscopic appearance of soft tissue invasion.*



- **Regional Lymph Node (N)**

Nx		Lymph nodes cannot be assessed
N0		No regional lymph nodes metastases
N1		Metastases in a ipsilateral lymph node measuring ≤ 3 cm in the greatest dimension
N2	**N2 a**	Metastases in a ipsilateral lymph node measuring between 3 and 6 cm in the greatest dimension
	N2 b	Multiple ipsilateral lymph node metastases measuring none of them more than 6 cm
	N2 c	Bilateral or contralateral lymph node metastases measuring none of them more than 6 cm
N3		Lymph node metastases measuring ≤ 3 cm in the greatest dimension

- **Distant Metastasis (N)**

M0	No distant metastasis
M1	Distant metastasis

➢ 16. 1. 3. ANATOMIC STAGE

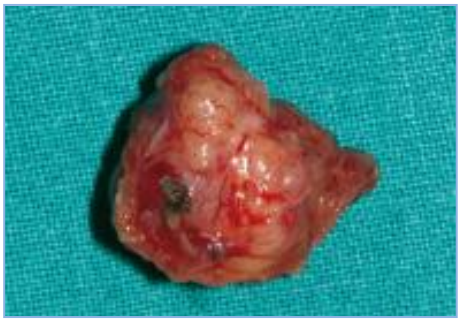
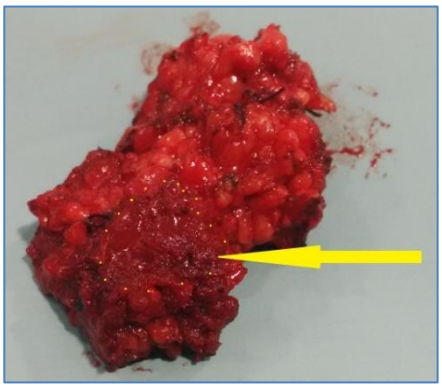
Stage I		T1	N0	M0
Stage II		T2	N0	M0
Stage III		T3 T1- T3	N0 N1	M0 M0
Stage IV	**A**	T4a T4a T1 – T4a	N0 N1 N2	M0 M0 M0
	B	T4b Any T	Any T N3	M0 M0
	C	Any T	Any N	M1

Reference:

(30)(31)(32)

16. 2. ANNEX II: RANKOW CRITERIA (6)(8)

Regardless of the histological tumour type, there are some criteria (*Rankow*) that suggest the presence of a benign or malignant tumour:

	RANKOW CRITERIA	
	BENIGN	**MALIGNANT**
Duration	years	months
Gender	female	female
Pain	unusual	common
Facial nerve paralysis	rare	20 – 30%
Mobility	yes	no
Consistency	firm	stony
Stones	occasionally	rare
Gamma-scan	Warthin	no
	Figure A : *Plemomorphic adenoma* ***Author:*** (33) 	***Figure B:*** *Superficial parotidectomy Arrow is showing an adenocarcinoma.* ***Author:*** *Marta Valls Gay* 

16. 3. ANNEX III: DATA COLLECTION

- **FNAC pathology process**

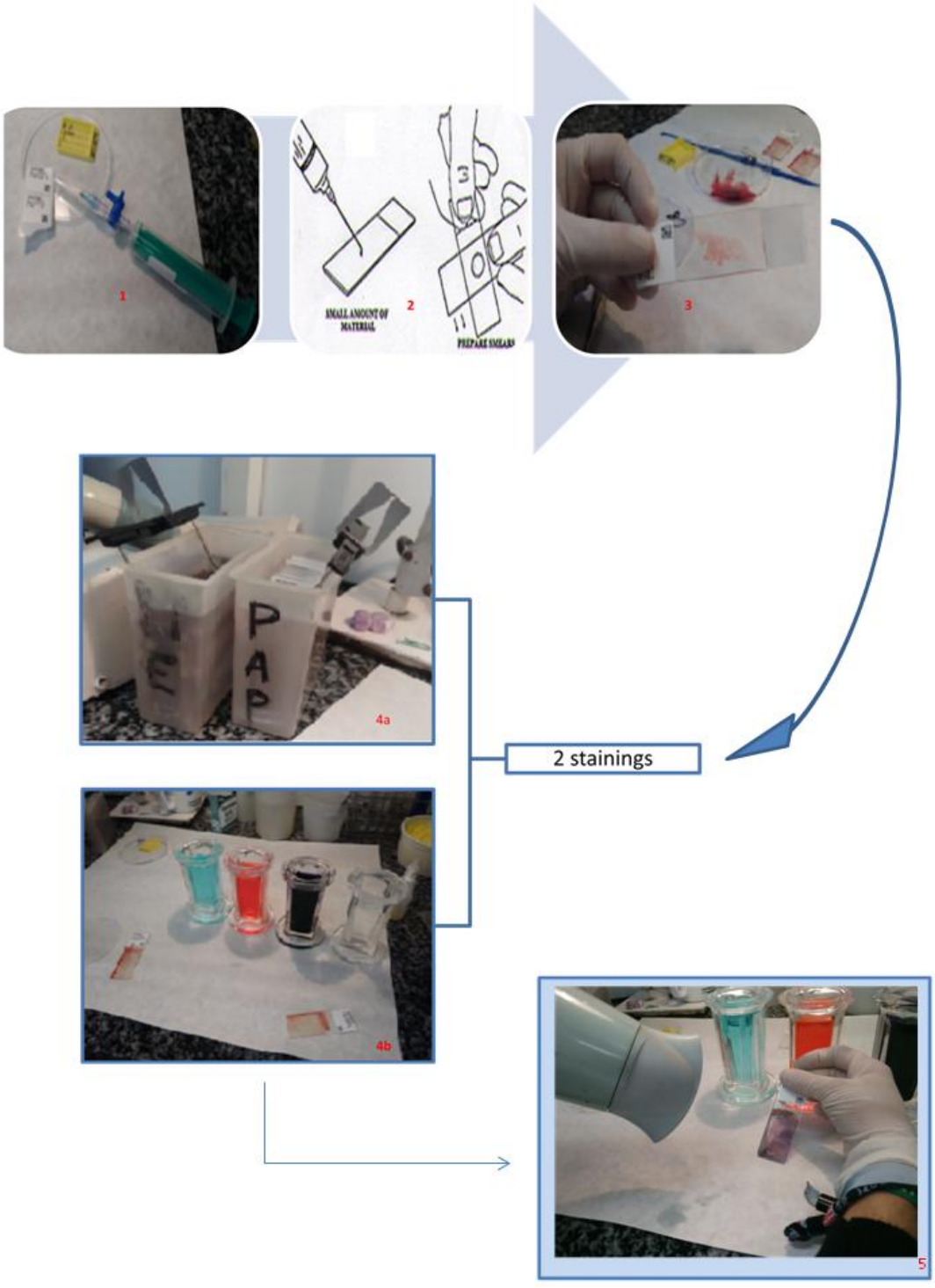


Figure : *FNAC procedure *Detailed steps on Section 8.6: Data collection*
Author: *Marta Valls Gay (images 1 , 3, 4a, 4b, 5), Carlos Zamorano and Julieta Sepúlveda (image 2)*

16. 4. ANNEX IV: INFORMED CONSENT

Hospital Universitari de Girona Doctor Josep Trueta

CONSENT FORM

Project title: *Role of Fine-Needle Aspiration Cytology in the Diagnosis of Parotid Tumours: a Cross-Sectional Study*.

Patients name and surname: _____

Telephone number: _____ **Date of Birth:**_____

1. I confirm that I have understood the information sheet of the study and that the information is clear enough.
2. I understand that my participation is voluntary and that I am free to withdrawal it any time without giving any reason, keeping my medical care and legal rights.
3. I have expressed my misgivings in order to better understand the project.
4. I give permission to study researchers and collaborators to have access to my medical notes and data collected during the study.
5. I voluntarily agree to take part in this study.

Patients signature: **Doctors signature:**

Patients name: Doctors name:

_____ _____

DATE __ / __ / ____ **DATE __ / __ / ____**

16. 5. ANNEX V: CASE REPORT FORM

Hospital Universitari de Girona Doctor Josep Trueta

CASE REPORT FORM

Project: *Role of Fine-Needle Aspiration Cytology in the Diagnosis of Parotid Gland Tumours: a Cross-Sectional Study*

Patients name, surname and number of clinical history must not appear in this document.	**Identification number:** _____	**Sex:** □ Male □ Female	**Date of birth:** __ / __ / ____

Oral and Maxillofacial Surgery specialist: _____

Licence number: _____

VISIT 1	**Day:** __ /__ / ____	**Clinical diagnosis:** _____
VISIT 2	**Day:** __ /__ / ____	**FNAC diagnosis:** _____
VISIT 3	**Day:** __ /__ / ____	**HDFSS diagnosis:** _____ **Dimension of the tumour (mm^3):** _____

Additional commentaries:

16. 6. ANNEX VI: INFORMATION SHEET

Hospital Universitari de Girona Doctor Josep Trueta

INFORMATION SHEET FOR PATIENTS

Project title: *Role of Fine-Needle Aspiration Cytology in the Diagnosis of Parotid Tumours: A cross-sectional study*.

Main Researchers: Manel Gorina Faz, Marta Valls Gay

We are writing to you to inform and invite you to participate in a research study that is being held in the Universitary Hospital of Girona Doctor Josep Trueta. Before you decide whether or not you wish to participate in this study, it is important for you to understand the research is being done and what it will involve. Please take your time to read the following information carefully and ask us if there is anything that it is not clear of if you would like more information.

This study has been approved by the clinical research ethical committee in accordance with current legislation, and it is performed with respect to the principles mentioned in the Declaration of Helsinki and to the standards of good clinical practice.

Volunteer cooperation: Participation in this study is totally voluntary. If you decide to participate you will be asked to sign a consent form. If you decide not to take part of the study it will not affect the standard of car you receive. Once you are in the study you can withdraw your consent in any moment.

Purpose of the study: Fine- Needle Aspiration Cytology (FNAC) has been widely used as a diagnostic tool in clinical practice and it is a well-established tool for investigation many head and neck conditions. Its application in Parotid Gland Tumours (PGTs) is however controversial: preoperative diagnose of parotid cancers remains an up-to-date issue in hospitals and still generates discrepancies among physicians. However, a false diagnosis for neoplasia can result a delay of treatment and progression of a disease.
In this sense, giving medical evidence of a reliable and accurate approach that provides adequate information on what concerns preoperative diagnosis is the endpoint of this project, because effective treatment for PGTs starts with accurate preoperative diagnose.
The aim is this study is to evaluate the sensibility and specificity, positive and negative predictive value and accuracy of FNAC in the detection of parotid tumours.

Confidentiality: The information that we collect for this research project will be kept confidential in accordance with *Organic Law of Data Protection (15/ 1999)*. The data will be used only for purposes of this study. Any information about you will have a number on it instead your name.

Thank you for reading this. If you agree to enter the study,
please sign the attached consent form and we will return a copy to you

16. 7. ANNEX VII: CONTINGENCY TABLES. SENSITIVITY, SPECIFICITY, FALSE POSITIVES, FALSE NEGATIVES AND ACCURACY FORMULE

		HDFSS		
		Malignant	**Benign**	**TOTAL**
FNAC	**+**	a	b	**Total +:** (a + b)
	-	c	d	**Total - :** (c + d)
		Total malignant: (a + c)	**Total benign:** (b + d)	**Grand total:** (a + b + c+ d)
		a= True positive (TP): positive FNAC results on malignant tumours **b=False positive (FP):** positive FNAC results on benign tumours **c= False negative (FN):** negative FNAC results on benign tumours **d= True negative (TN):** negative FNAC results on benign tumours		

- **Sensitivity (S):** It is also known as true positive rate. It measures the proportion of positives that are correctly identified as such.

$$S = \frac{a}{a+c} = \frac{TP}{TP+FN} = \frac{people\ with\ positive\ FNAC}{total\ malignant}$$

- **Specificity (SPC):** measures the proportion of negatives that are correctly identified as such.

$$Sp = \frac{d}{b+d} = \frac{TN}{TN+FP} = \frac{people\ with\ negative\ FNAC}{total\ benign}$$

- **Positive predictive value (PPV):** measures the proportion of true positives in all the positive results of the diagnostic test.

$$PPV = \frac{a}{a+b} = \frac{TP}{TP+FP}$$

- **Negative predictive value (NPV)** measures the proportion of true negatives in all the negative results of the diagnostic test.

$$NPV = \frac{d}{c+d} = \frac{TN}{TN+FN}$$

- **Accuracy (A):** measures the proportion of valid results of the grand total

$$A = \frac{a+d}{a+b+c+d} = \frac{TP+TN}{TP+TN+FP+FN}$$