Universitat de Girona
Facultat de Medicina

VALIDATION OF SENTINEL LYMPH NODE BIOPSY IN EARLY STAGES OF OROPHARYNGEAL CANCER

FINAL DEGREE PROJECT

PAU DENGRA PEREZ

Clinical tuthor: Marc Tobed Secall and

Ignasi Sambola Cabrer

Methodological tutor: Abel López Bermejo

I would like to express my sincere gratitude to all those who have accompanied me and contributed to this work. Especially to Dr. Marc Tobed for agreeing to mentor me and to Dr. Ignasi Sambola for introducing me also in the specialty of otorhinolaryngology.

I would also like to express my thanks to Dr. Abel Lopez for the follow-up and support given throughout this work.

1. ABREVIATIONS

CT	Computed tomography
ECS	Extracapsular spread
END	Elective neck dissection
H&E	Hematoxylin-eosin
HPV	Human papillomavirus
IHC	Immunohistochemistry
MRI	Magnetic resonance imaging
PET	Positron emission tomography
RT	Radiotherapy
SCCHN	Squamous cell carcinoma of the head and neck
SLN	Sentinel lymph node
SND	Selective neck dissection
SSS	Step serial sections
US	Ultrasonography
UsgFNAC	Ultrasonography guided fine needle aspiration cytology

2. ABSTRACT

Background: Over the years the approach to cervical lymph node metastasis in oropharyngeal cancer has evolved to a more conservative form. The controversy arises in cases where it is not possible to detect cervical lymph node metastasis by means of clinical or imaging techniques. In these cases, elective neck dissection is traditionally recommended when the tumor size and subsite confers at least a 20% risk of lymphatic spread. This implies that a high percentage is submitted to a surgical procedure without need. Therefore, elective neck dissection is the current treatment and gold standard for neck staging. The sentinel lymph node biopsy is a promising technique that its application in clinical practice could imply the saving of an unnecessary surgical intervention. In the same way that it could play an important role in lymphatic staging as well as in prognosis, since lymphatic involvement is the main risk factor for decreased survival.

Obejective: The main objective is, on one hand, to determine the sensitivity, specificity and negative predictive value for sentinel lymph node biopsy validation and, on the other hand, to determine the recurrences and survival in the same group of patients in order to reinforce the validity of the sentinel lymph node.

Methods: A cross-sectional study will be conducted to establish the diagnostic test validation. A sample of 32 subjects selected in a consecutive non-probability manner will be analyzed.
Once the lymphatic staging has been performed, the sample will be included in a secondary study. A longitudinal study will be carried out dividing the sample between two groups depending on the lymph node involvement. These two groups will be followed to evaluate local and regional recurrences at 2 years, as well as survival at 5 years. We will use Cox regression for the multivariate analysis.

Key words: Oropharyngeal cancer; sentinel lymph node biopsy; occult lymph node metastasis; recurrences; prognostic

3. INTRODUCTION

3.1 Background

Head and neck cancer is a term that includes the epithelial malignancies located in the paranasal sinuses, nasal cavity, oral cavity, pharynx and larynx. Almost all of these epithelial malignancies are squamous cell carcinoma of the head and neck (SCCHN).
For the interest of this work, we are going to be focus on the oropharynx, as some characteristics are common between other locations of the head and neck cancer.

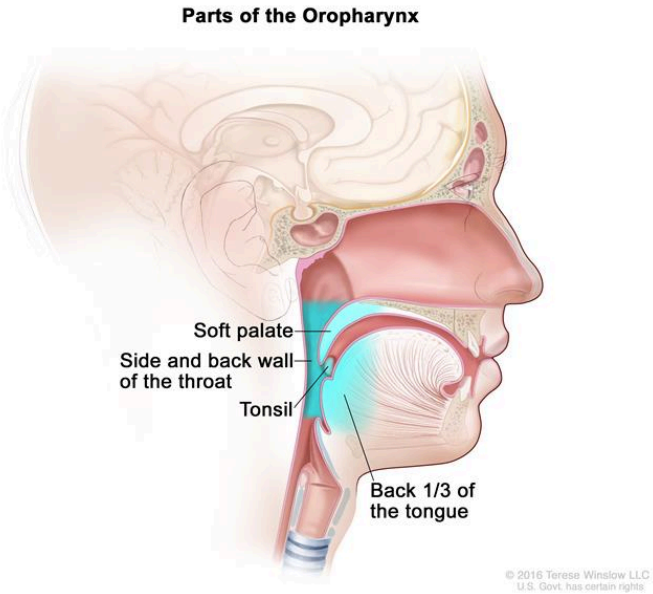
First of all we have to consider that most epidemiology reports, cancers of all sites of the oral cavity (lip, tongue and mouth) and pharynx are grouped together so in this work are cited as they were originally described.

Worldwide the cancer of the oral cavity and the pharynx is the 7th most common type of cancer. With an estimated 529000 new cases in 2012 and with 292000 deaths is the 9th most common cause of cancer death. But, when the main subsides (lip, oral cavity and pharynx) are examined separately, they don't rank highly. (1)
In Europe oral and pharynx cancer have an annual incidence of 18.2 in males and 4.9 in females. There are differences in the incidence between countries of West Europe and East Europe, yet the main difference is in the mortality. In 2012, the age-standardized cancer mortality among men was 5.1 in Central and Eastern Europe and 1.6 in Northern Europe, these differences could be explained through their socio-economics characteristics and the limited treatment facilities in countries from East Europe. (2)
In Spain, the incidence of oral and pharyngeal cancer in 2012 was of 10.1 new cases for 100000 persons, which means 5978 new cases. Divided by gender the incidence in males was 16.8 and in females 4.2. The mortality in Spain in 2012 had a total number of 2070 (3.4) persons and divided by gender it was also higher in males (5.8) than females (1.3). (2)

The issue with the registers for the oropharyngeal cancers is that the majority of them, independently of what target population they have, are grouped with the rest of the pharyngeal cancers, or more frequently with the oral cancer, which can generate a confusion in the trends of incidence and mortality. That can be explained by the risk factors associated, while in the SCCHN historically the main risk factors have been the alcohol and the tobacco, in the oropharynx, the Human papillomavirus (HPV) has taken an important role as another risk factor together with the alcohol and the tobacco. That explains the decreasing incidence trend for the oral and pharyngeal cancer as the tobacco and the alcohol consumption have reduced over the years, but when the oropharyngeal cancer is studied alone there is not a decrease of the incidence as a result of the increasing HPV prevalence.
That trend has been seen in developed countries, such as the United States where there has been most of the epidemiologic research about this topic. (3)

3.2 Oropharyngeal cancer

The oropharynx is the middle compartment of the pharynx and includes the tonsils, tongue base (posterior 1/3), soft palate and pharyngeal walls.



Parts of the Oropharynx from the National Cancer Institute

The squamous cell carcinoma represents 91% of all the primary tumours of the oropharynx and we can find three main risk factors.

- Tobacco: Is the main risk factor, it increases with cumulative smoking, the more years and more cigarettes per day higher is the risk. (4)

- Alcohol: The increase of the risk is also associated with a higher consumption of alcohol. It has an interaction with smoking, the joint effects of having both behaviours are grater than multiple of the effects of the individual behaviours, this joint behaviour is found in 72% of pharyngeal cases. (4)

- Human papillomavirus: Over the past years we have seen a decrease in the smoking rates, however there has been an increase in the incidence of oropharyngeal squamous cell carcinoma. This increase is now attributed to HPV and is expected that it will continue to grow. Transmission of HPV is primarily through sexual contact and oral-genital contact. The majority of HPV-related oropharyngeal carcinoma cases are caused by HPV16, detected through immunohistochemical (IHC) analysis.

  In the daily clinical practice it's important to know that not all squamous cell carcinoma of the upper track occur in heavy smokers and drinkers. The HPV-related patients often presents itself in younger ages, without a history of heavy smoking or drinking but with a history of different sexual partners. The HPV positive oropharyngeal squamous cell carcinoma is more likely to present itself with small primary tumors and more extensive nodal diseases. This makes the seek of treatment more common due to the nodal disease symptoms rather than the primary tumor symptoms.

  Survival in HPV positive patients has improved compared to HPV negative patients, despite presenting with advanced nodal disease. Also patients with HPV positive are less likely to develop second primary malignancies with an overall recurrences rates lower than the HPV negative patients.

However, HPV positive oropharyngeal carcinomas have a different pattern of distant metastasis. They have a higher proportion of recurrence at distant sites, and more likely to develop in non-traditional sites (other than lungs). These metastasis may develop more than 2 years after the initial treatment, whereas HPV positive typically occur within the first 2 years. In recent years, studies have been carried to determine if HPV positive patients should be treated with a more graduate treatment. (3) (5)

- 3.2.1 Diagnosis and staging of the primary tumor

The clinical presentation is often a painless neck mass with few other symptoms associated, such as sore throat or tongue, otalgia, difficulty swallowing and/ or change in voice quality (hot potato voice). In some cases, patients may also complain about pain.

Clinical examination is preformed with flexible direct endoscopy of the upper aerodigestive tract to determine the lesion and the localization in the oropharynx.

For the staging of the primary tumor, magnetic resonance imaging (MRI) scanning with contrast is the optimal imaging test, particularly in the soft tissue affections (e.g. tongue). Computed tomography (CT) scanning may also be required, it has a value in the study of the nodal disease and bony invasion.
Distant metastases should be assessed by CT scanning of the chest and upper abdomen to exclude metastatic disease to the lungs and liver. (6)
Fluro-dexoy-glucose positron emission tomography combined with CT (FDG PET-CT) scanning could give valueable information in cases that the full tumor extent is not clear because of the diffuse infiltration. The PET/CT has the best indication when the patient is presented with cervical lymph node metastasis without being able to find the primary tumor. The results of the PET/CT on these patients determines a more conservative or aggressive treatment.(7)

The definitive diagnostic is histologic, tumors can be biopsied under local anaesthetic in the clinic. Otherwise, direct biopsy and general anaesthetic is necessary. The vast majority, as mentioned before, are squamous cell carcinomas.

According to recent publications about the implication on the prognosis, HPV p16 protein testing is a core item for the oropharyngeal carcinoma. Even the presence of HPV has an implication in the staging of the tumor, as it's shown in the last edition of the TNM Classification (8th edition, 2018) for oropharyngeal tumors (Annex 2) (5) (8)

The IHC identification of over-expression of p16 protein is a useful screening method for HPV infection as HPV-related oropharyngeal carcionoma show a cytoplasmatic expression of p16 protein over 70 per cent of malignant cells. If possible, HPV-related carcinomas should have the presence of HPV confirmed by HPV DNA in situ.

- 3.2.2 Treatment of early disease (T1-T2)

Both surgery and radiotherapy (RT) provide similar locoregional control and survival control. The choice depends on the functional outcome, the patients general condition, the possibility of an adequate follow-up and the probability to develop a second primary tumor (e.g younger age with risk factors associated, in that case is preferred a surgery treatment).

Radical RT is a good option, a total dose equivalent of 70Gy in 35 fractions is used. Usually, surgery should be carried transorally, either by transoral laser microsurgery or transoral robotic surgery. By use of these modern surgical techniques, the surgical excision can be achieved with functional preservation of much of the involved organ and good oncologic results. There is no uniform unresectability criteria, but most of the surgeons accept invasion of the carotid artery, base of the skull or paravertebral musuculature as unresectable.(9)(10)

Although the goal for T1-T2 desease should be single modality treatment, adjuvant RT and/or chemoradiothearpy may be required due to adverse pathological features for recurrences following surgery.

Patients with extra-capsular invasion and/or involvement (<1mm) of the surgical margins around the primary tumor will benefit most from adjuvant treatment. RT should be planned using the same principals as radical RT. (6)

3.3 Neck staging

The neck metastases in the head and neck tumors have the biggest impact in the prognosis of the patient. (11) Controversy surrounds the management of the neck in the SCCHN due to multiple studies of high-level evidence for many treatment paradigms, especially around the occult metastasis and the complexity of the distributions and the patterns of the lymph node metastasis.

- 3.3.1 Diagnosis of lymph node metastasis

The diagnosis of nodal involvement could be clinical by palpation of the neck, though is regarded as inaccurate (sensitivity and specificity 70-80 per cent), due to the variability of the operator, the shape of the neck, the subcutaneous fat of the neck and the varying size of the involved cervical nodes.

The radiological techniques that are more used in daily practice are the CT and the MRI. These two techniques have a similar sensitivity (81 per cent) but the CT has a better specificity (76 per cent) compared to the MRI (63 per cent). The main advantage is the availability in almost all hospitals and also that a general radiologist could interpret it. (12) (13)

This is compared to the ultrasonography (US) and the ultrasonography guided fine needle aspiration cytology (USgFNAC). These are two techniques with a high operator dependency. However, R.B.J de Bondt et al. reported in a meta-analysis that the USgFNAC has the best diagnostic performance for the detection of cervical

lymph nodes with a sensitivity of 80 per cent and a specificity of 98 per cent. Also noticed the US had a better performance than the CT and the MRI with a sensitivity of 87 per cent and a specificity of 86 per cent. But, the US and the USgFNAC are not popular tools in the daily practice in our environment due the high operator dependency as well as the often need to be performed by experienced specialists in referral hospitals. (14) It must also be said that the tendency in our region is for US use to spread between hospitals and specialists in charge of diagnosing oropharyngeal cancer.

There is not a consensus with the criteria to evaluate the node and differentiate between benign and malignant. Is important to differentiate that in the US, the criteria is used to discriminate among malignant and benign, while in the USgFNAC is used to decide whether to puncture or refrain from puncturing a lymph node.
Most studies use the following general criteria to differentiate between benignity and malignancy(14)(15):

US	CT/MRI
– Size > 8-10mm – Hypoechogenicity relative to adjacent musculature – Contour irregularity (sharp borders) – Round shape – Absence of echogenic hilium – Peripheral or mixed vascularity by Doppler	– Size > 10mm – Contour irregularity – Intern heterogeneity – Necrosis – Rim enhancement

For the staging of the neck, prior to the treatment plan, the N category of the TNM classification (8th edition) shown in Annex 2 is used.

The main concern is with the cases which these imaging tools cannot detect the presence of lymph node metastases due to the high risk of occult metastases.

Furthermore, because of this risk, the imaging alone may not be accurate enough to guide specific treatment decisions. (16)

The PET/CT is not recommended routinely to assess possible cervical lymph node metastasis in cN0 stage due to the low specificity to differentiate the inflammatory reactive nodes and the adjacent granulation tissue from the metastasis. Also, in these cN0 patients, the PET/CT have a low sensitivity due to the size of occult metastasis that is beyond resolution of the PET (typically micrometastasis). So, the PET/CT doesn't give added information to the CT or MRI.(7)

- 3.3.2 Pathology evaluation

The most reliable way to determine the nodal metastasis is after the elective neck dissection (END) with the pathologist analysis; therefore it's the current gold standard to establish the nodal infiltration of tumor cells.

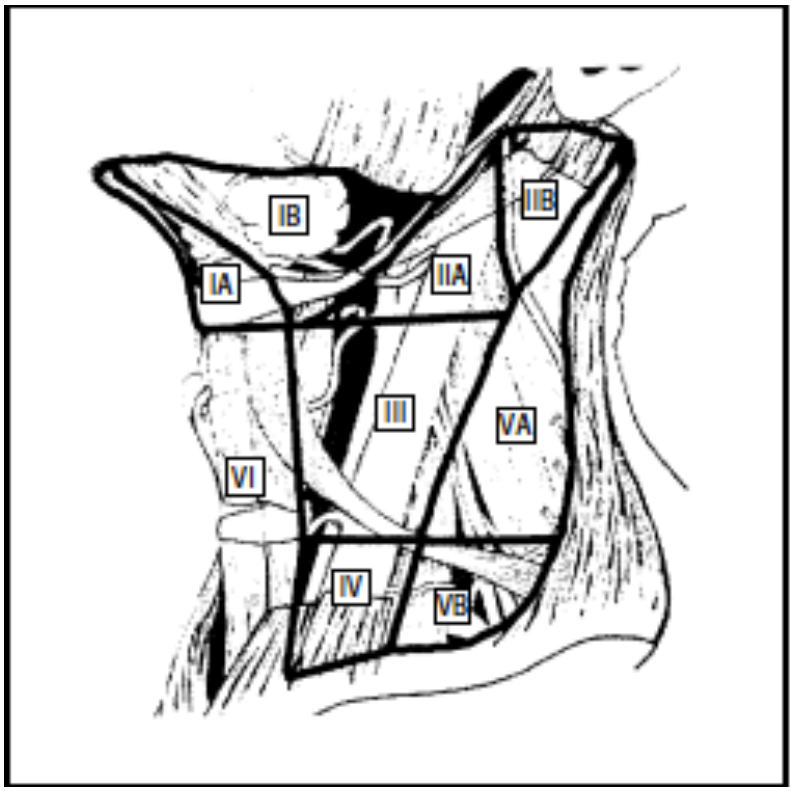
The nodes dissected should clearly state which nodal groups are included and should be clearly orientated. Fixation is in a formaldehyde-based solution for 24-48hours. Each discrete node is dissected out with attached pericapsular adipose tissue. If there is obvious metastatic tumor, the half/slice with the more extensive tumor should be processed, together with the perinodal tissue to show the extent of extracapsular spread (ECS). If the node appears negative, all slices should be processed. One hematoxylin-eosin (H&E) stained section from each block is usually sufficient for routine assessment. ECS is associated with a poor prognosis and any spread through the full thickness of the node capsule is regarded as ECS which should imply the use of adjuvant RT. (17)(18)

- 3.3.3 Division of lymph nodes by levels

To delineate the location of lymph nodes in the neck, a level system is used dividing the neck in 6 levels. Some of them may have biological independence of the larger zone in which they lie, so those are divided as sublevels. This description

is extracted from the consensus of the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS) in Annex 1 (19)(20)

Depending on the localization of the primary tumor, we can establish which neck levels have a higher risk of being affected by dissemination of the tumor cells through the lymphatic system. In the oropharyngeal cancer, the consensuses for lymph nodes removal are the ones located in levels II to IV. (19)



The 6 sublevels of the neck from Robbins et al. (19)

Apart from the neck levels, in the retropharyngeal space we find a group of lymph nodes that receive an afferent lymphatic drainage from the pharynx, with an efferent drainage towards the upper jugular space.

These retropharyngeal lymph nodes (RPLN) are of clinical importance. The first to describe the relationship between its involvement and prognosis was Ballantyne et al. (21). The debate arises in the diagnosis and management of these nodes, since

END does not include dissection of the RLPN and diagnostic techniques cannot determine if there is hidden metastasis.

We cannot find many studies that establish the true incidence of metastasis in the RLPN because their evaluation in most studies is only radiological. Therefore, there is a percentage of hidden metastases that cannot be accounted for.
In a retrospective study, with 981 participants and with oropharyngeal cancer was established by radiology an involvement of RPLN of 10%. It was also associated with a reduction in 5-year survival, a decrease in local control and lymph node control. Similar percentages of involvement were found in other studies with a clear association with decreased prognosis. (22)(23)(24)
In clinical practice, this risk must be taken into account and represents a diagnostic challenge in which the sentinel lymph node could play a significant role.

3.4 Management of the N0 neck (25)

The risk of occult metastasis in these patients must guide the therapeutic decision. The issue is the lack of evidence to determinate a specific criteria, with a high predictability, to define the risk of occult lymph node metastasis, which would allow to decide with high confidence which patients would need an elective treatment of the neck and which would be subjected to "wait and see" policy.

The current guidelines stipulate that those patients with a risk above 20 per cent of occult node metastases will need an elective neck dissection, even though we can affirm that there will be occult metastasis involved in the neck.
In order to take this decision, the factors that have a predictable value for occult neck metastasis are (26) (27):

- Tumor thickness and depth (≥4mm)
- Muscle invasion
- Poorly cell differentiation
- Perineural invasion
- T2 stage with HPV p16 postive

- 3.4.1 Surgery

Historically, the mainstay of surgical treatment of metastatic neck consisted in the neck dissection in its various forms. (19)

- Radical neck dissection (RND): Removal of all ipsilateral cervical lymph nodes, including levels I through V, spinal accessory nerve, internal jugular vein and sternocleidomastoid muscle.
- Modified radical neck dissection: Consists in the excision of all lymph nodes, routinely removed by the RND with preservation of 1 or more nonlymphatic structures (spinal accessory nerve, internal jugular vein, sternocleidomastoid muscle)
- Selective neck dissection (SND): Refers to a cervical lymphadenectomy in which there is a preservation of 1 or more groups removed in the RND.

The tendency over the years is clearly to be the least invasive as possible. The classic RND has no role in the elective treatment of the N0 neck. The choice should be SND at the same time as the primary tumor is resected.
Several studies support the elective treatment of the neck with SND over the "wait and see" with strict control followed with a therapeutic neck dissection. A randomised controlled trial with 596 patients enrolled, with lateralized T1- T2 oral squamous cell carcinoma, proved that SND has higher rates of overall survival and disease-free survival. (28)

Even if the SND is considered the least invasive procedure we have to contemplate that this procedure involves some complications. We must have in mind that the majority of these patients considered to have a risk over 20 per cent of occult metastasis, will be submitted to this surgical procedure with no evidence of lymph node metastasis. So, no depreciable number of N0 patients will be treated unnecessarily, because a lot of them will still be staged as pN0 after the END.

Among the complications of the SND we can find (29):

- Shoulder drop due to spinal accessory nerve injury.
- Stiffness and constriction of the neck
- Pain and numbness caused by the manipulation of the cervical nerve during the level IV neck dissection.

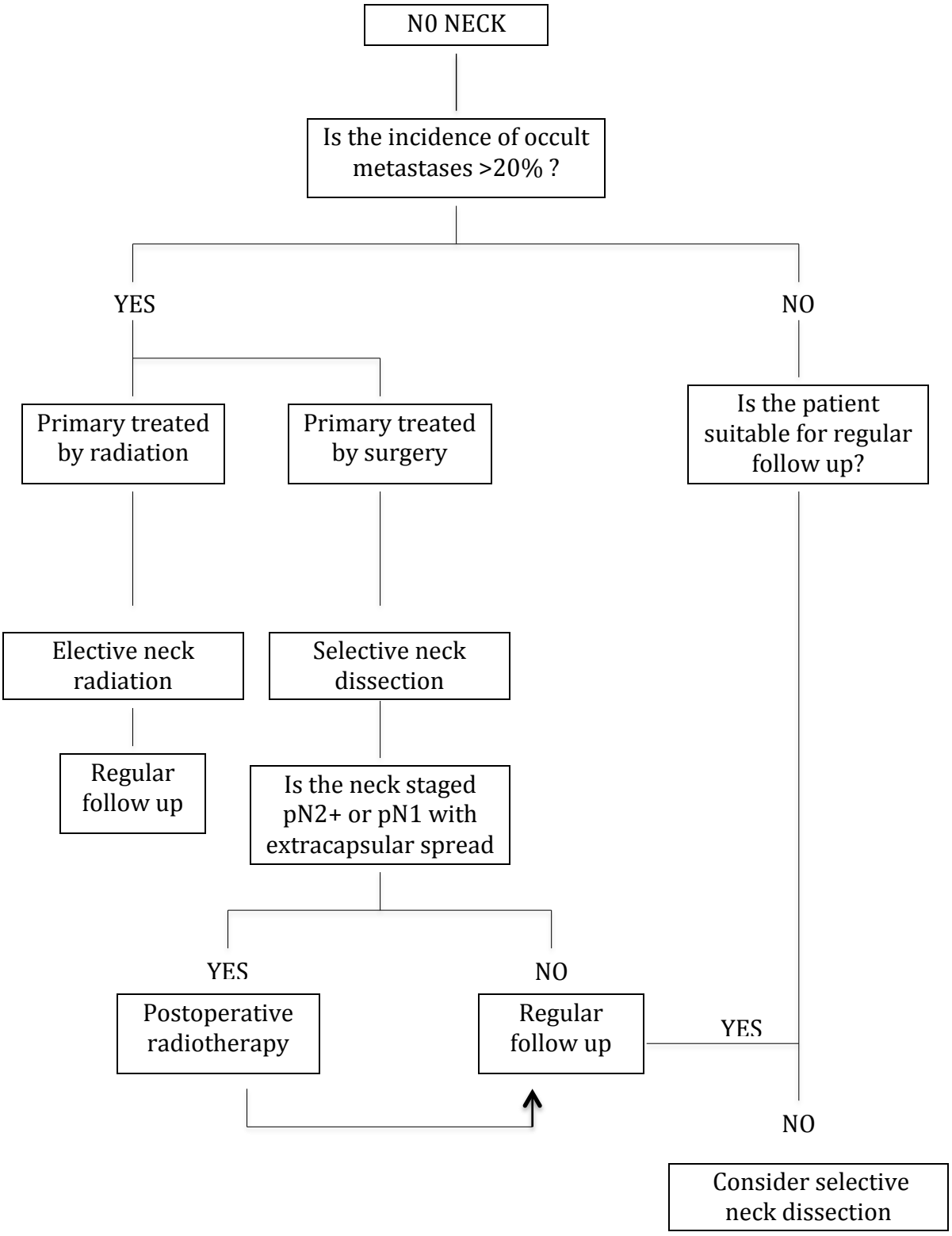
Another aspect of debate, is the decision to treat the neck bilaterally with END of both sides. Not all cases need a bilateral neck dissection, but large retrospective series have reported on the risk of contralateral involvement.
The current data and guidelines recommend that a neck staged N0 should be treated when the primary tumor is close to the midline. Especially the cases that the tumor in the oropharynx is located in the pharyngeal wall or in the base of the tongue; and/or in some cases, when they are T2 stages, independently to the proximity of the midline, for example the carcinomas of the soft palate. (30)
The sentinel lymph node technique would be very helpful in this decision, providing accurate information in order to select those patients that need a bilateral neck dissection.

- 3.4.2 Radiotherapy

Radiotherapy has an important role in the management of the N0 neck as wll. Elective neck radiation has similar results to END, but it's preferable when the primary tumor is treated with radiotherapy. The first nodes close to the primary tumor are the ones with a higher risk of occult metastases, and are usually included in the high dose or radical radiotherapy treatment volume. The RT treatment should be delivered in an accredited department using intensity-modulated radiotherapy.
After SND, patients staged pN1 with extracapsular spread or as pN2 (or greater) should be treated with postoperative radiotherapy to reduce the risk of recurrence. (31)

Patients with a risk lower than 20 per cent that are suitable for follow up or patients after END / RT, should be subject to regular control with neck examination and US / USgFNAC surveillance by expert radilogists. Regular check-ups should be with high-frequency intervals during the first two years, when the risk of localregional recurrence is high, followed by a decrease in frequency after the second year.

Therefore the follow-up of the first two years should be every 16 weeks and every six months the following years. Patients follow-up should last up to 5 years but longer periods can be justified for high-risk patients. (32)(33)



Algorithm for management of the N0 neck adapted from United Kingdom National Multidisciplinary Guidelines (25)

3.5 Sentinel lymph node biopsy

This concept states that the sentinel lymph node (SLN) is the first node that the tumor will metastasize via the lymphatic system. After this first-draining lymph node or nodes, the tumoral cells will spread to the rest of the regional lymph nodes. So, the pathologic status of the SLN should accurately reflect the histology of the remaining lymph nodes, and neck dissection will only be required when the SLN is affected.

This technique is based on the lymphatic spread pattern of the tumor. Furthermore, it's important to know that this lymphatic flow is different between localizations of the head and neck tumors.

The first reported case of a patient with neck metastasis identified by the SLNB in a SCCHN, was published in 1996 by Alex and Krag for a patient with supraglotic cancer. (34) The continuous controversy around the cN0 patients and the risk of occult metastasis with the resulting recurrences, justified the interest for this technique and further investigations were conducted to formulate a method for the procedure. Shoaib et al. conducted a study in patients undergoing neck dissection and compared the use of blue-dye alone versus blue-dye and radiolabeled Tc99. The results clearly showed that the group of patients with blue-dye and radiocolloid had a better percentage of SLNs identified. (35)

Radiocolloid

The ideal radiocolloid should be able to selectively identify the sentinel lymph node and should remain trapped to reduce the non-sentinel node background emissions. Larger particles colloids are less likely to pass to the lymphatic channel and penetrate to the SLN. For the SLNB the radiocolloid most frequently used in our environment is the Tc-99m-labeled human serum albumin colloid (Nanocoll). It performs satisfactorily in all tumor types studied. Nanocoll migrates to sentinel node within minutes, yet prolonged retention allows surgery to take place the day following.

Tracer injection should be at 0.1-0.5 cm from the tumor o scar margin. The traced should be administered on each side of the tumor/scar. For lesions in sites with abundant soft tissue, four separate injections must be given around the lesions. For lesions located in muscle (i.e tongue), injections should be performed according to the depth of the lesion. (36)

The use of blue-dye is optional. However, when used it is a useful adjunct to aid SLN localization and harvest. Blue-dye drains to the SLNs via the same lymphatic pathways as radiocolloid. It may aid the surgeon with direct visualization and dissection of SLNs.

Lymphoscintigraphy

An essential prerequisite for successful SLNB procedure is an accurate map of the pattern of lymphatic drainage from the primary tumor site. The role of lymphoscintigraphy is to provide such a map in each patient. This map should indicate not only the location if all SLNs but also the number of SLNs at each location. Lymphoscinitgraphy uses a gamma camera to asses the drainage of injected radiotracer via the lymphatic capillaries until it either passes through, or until is retained within, the regional lymph nodes.(37)

Pathology evaluation

The use of the SLNB allows the pathologist to use more exhaustive techniques because only a few specific lymph nodes will be necessary to be evaluated.
The routine H&E stain has the inconvenient that it may skip the micrometastases (2mm or less in diameter but greater than 0.2mm) and the isolated tumor cells (<0.2mm in diameter). In order to detect them a more exhaustive analysis is necessary, with H&E at 150µm step serial sections (SSS) and/or immunohistochemistry (IHC) with anti-cytokeratin antibodies AE-1/AE-3. (38)(17)

Whether the presence of occult metastases is of clinical significance or not, is still under debate. Some authors suggest that they might indeed have some prognostic value. (18) For example Jung-Hae Cho et al. in a retrospective study reported that the presence of micrometastases could predict the 5-year survival rate, associated with poor prognosis compared with the patients that didn't have micrometastases, although they also found an association between the depth of invasion of the primary tumor and the micrometastases. (39)
The detection of micrometastases could represent an improvement of the survival due to the reduction of the recurrences, which would imply a treatment with RT for those selected patients.

Thompson et al. designed one of the largest meta-analyses to evaluate the diagnostic value of the SLNB, with 26 publications reporting an overall sensitivity and negative predict value of 95% and 96% respectively. In all the studies the patients had a concurrent END performed at the time of the SLNB. Although, the aim of this meta-analyses was to include all of the head and neck cancer localizations, the majority of patients in the studies had oral cavity tumors.
Within the 26 studies analysed, 72 patients had oropharynx cancer and these studies showed similar percentages of sensitivity and negative predict value to the overall results. The researchers concluded that, in oral cavity tumors the SLNB is a valid technique to correctly stage regional metastasis. (40)

In oral cavity tumors, several institutions and some guidelines apply this technique in the N0 patients for the correct staging of the neck, as it is the most studied localitzation.

For the oropharynx, further studies are necessary to conclude the validity of the SLNB but very promising results have been reported.

4. JUSTIFICATION

The management of the neck for head and neck cancer has been controversial from the beginning. The lymph node metastasis is the main prognosis factor, and the correct diagnostic and management have a direct impact in the survival of the patient. Neither clinical examination nor imaging are accurate enough to guide the management in a reliable manner, due to the difficulty to detect occult metastasis, which in several studies, rated values between 10-25 per cent. (6)(16)

For this reason, the management of oropharyngeal cancer that is staged clinically and radiologically as N0 has been a continuous point of debate.

Nowadays, there is no way to affirm that a N0 neck does not face the risk of suffering a recurrence. This is why, when the tumor size and subsite confers at least a risk over 20% of occult metastases, it is recommended that patients should be subjected to END. This implies a no depreciable number of patients that will probably be overtreated with a surgical procedure, with its associated morbidity.

The SLNB is a less invasive technique, which has proved its validity in other cancers, and could have an important role in the management of the neck. This would allow a change in the daily practice. Since implementing SLNB instead of the END would represent a decreased morbidity, operating room time, and length of postoperatory stay.

In addition, with the current techniques of transoral robotic surgery, the deep approach of the neck is significantly improved. Although, in the END an open approach is still necessary, the dissection of the sentinel lymph node could be carried out in a transoral way at the same time as the primary tumor is resected.

In this manner we could even avoid an open cervical approach to obtain the sentinel lymph node.

There are several studies published with good results that have shown the validity of this technique to stage the neck. Even in some studies, END has not been performed when SLNB has been negative

They have also proven that the SLNB provides important information in cases that resulted positive. It allows to establish, with more reliability, which cases need to

have a bilateral neck dissection or if the neck dissection needs to include other levels that initially would not be dissected for the localization of the tumor.
In addition, the SLNB could be a technique that will allow the detection of metastasis in the retropharyngeal space, which is not included in the SND.

Despite all of this, and although some institutions perform this technique, it is not yet applied in the routine management.

The aim of this study is to show the applicability of the SLNB in oropharyngeal carcinomas staged by clinic and image as N0. In order to do that, we will determine the sensitivity, specificity and negative predict value (NPV) and evaluate the accordance with the END.

Furthermore, as oncologic patients and knowing that the lymph node metastasis is the most important prognosis factor, a follow-up of these patients is mandatory. We want to take advantage of the routine visits and collect data to perform a secondary study. The main objective will be to stipulate the percentage of local recurrences at 2 years and the survival rate at 5 years in the same group of patients included in the cross-sectional study.
This study is proposed as a secondary study, but its results will also give strength to the sensitivity and the NPV obtained from the SLNB. The

If the applicability of the SLNB in the Hospital Universitari Dr. Josep Trueta is proven, further studies avoiding the END in the negative SLNB cases will be justified.

5. QUESTION

Is it possible to implement the sentinel lymph node biopsy as a staging method for cervical lymph node metastasis in early stages of oropharyngeal cancer?
(T1-T2 N0)

6. HYPOTESIS

The SLNB has a high sensitivity and a high negative predictive value and allows to establish the lymph node involvement more precisely.

7. OBJECTIVES

Main Objectives:

- The main objective is to determine the sensitivity, specificity and NPV of the SLNB to detect the lymph node metastasis.

Secondary objectives:

- To identify the amount of lymphatic metastasis.

- See if the positive nodes, detected by SLNB, are included in the neck levels II, III and IV (SND in the oropharynx) or we can find positives nodes in other levels. In order to observe this, we want to evaluate which level of the neck the SLN is found.
  At this point, is also of interest to analyse if the SLNB has a role in detecting lymph node metastasis in the retropharyngeal space.

- Evaluate if the SLNB is able to detect those cases that have contralateral or bilateral lymph node metastasis.

- Evaluate if some covariables interact with the result of the SLNB.

7.1 SECONDARY STUDY

Main objective

- We will analyse local recurrences at 2 years of follow-up and the survival at 5 years between, upstaged (pN+) patients and the patients who remained at the same stage (pN0) after the SLNB-END.

Secondary objective

- Evaluate which covariables may influence the prognosis.

8. MATERIAL AND METHODS

8.1 STUDY DESIGN

A cross sectional study will be carried out to evaluate the diagnostic test. It will take place in the Head and Neck Unit of Girona's reference hospital. The unit is composed for otorhinolaryngologists, pathologists, radiologists, nuclear medicine physicians an oncologists.

The cohort of study will be follow in a prospective longitudinal study to evaluate the local control of the disease at 2 years and the survival at 5 years.

8.2 SAMPLE SELECTION

8.2.1 CROSS-SECTIONAL STUDY

A consecutive non-randomised sampling selection will be performed. All patients will be histopathologically diagnosed with squamous cell carcinoma of the oropharynx in early stages (T1-T2) and without regional metastasis in cervical lymph nodes (N0) that could be detected by clinic, US or by CT and/or MRI

Inclusion criteria

- Patients diagnosed with primary oropharyngeal cancer smaller than 4 cm (T1-T2) tributary to END.
- No regional lymph nodes metastasis (N0) detected by clinic, US or by CT and/or MRI.

Exclusion criteria

- Patients with neoadjuvant chemotherapy or radiotherapy criteria.
- Patients with previous radiotherapy or surgery of the neck that might modify the lymphatic drainage.

- Patients that according with the current guidelines have a risk of nodal metastases under 20% and are not tributary of END
- Prior head and neck cancer.
- Patients with SLNB contraindications (pregnancy, lactating women, allergy to the blue dye or Tc99 injected in the border of the tumor).
- Contraindication for surgical treatment.
- Patients with distant metastasis.

All patients will be informed about the study and invited to participate voluntarily. They will receive a document with all the information (Annex 3) and the informed consent document (Annex 4) that has to be signed if they are willing to participate.

8.2.2 SAMPLE SIZE

In a bilateral contrast, with a significance level (alpha) of 5% and a power of 80%, and assuming that sensitivity is very high, close to 95% (40)(41), we will need 32 subjects to perform this study.
The computations were carried out with the Prof. Marc Saez' software based on the library 'pwr' of the free statistical environment R (version 3.5.1).

In Hospital Universitari Dr.Josep Trueta, there are about 20 patients per year diagnosed with oropharyngeal cancer in initial stages that can potentially meet the inclusion and exclusion criteria.
We will need approximately 1 year and 7 months to recruit the sample.

8.2.3 LONGITUDINAL STUDY

The population studied are patients that participate in the cross sectional study and fulfil the inclusion and exclusion criteria.

A withdrawal criterium, specific for this secondary study, is the detection of a second neoplasm during the follow-up.

The patients, in which the sentinel lymph node can't be located during the surgery and are only submitted to SND, will also be included in the study. According to other studies, we anticipate that there will be a low percentage of patients in which the SLN will not be found during surgery.

8.3 VARIABLES

8.3.1 CROSS-SECTIONAL STUDY

Principal outcome variable:

The principal variable is the neck lymph node involvement by malignant cells diagnosed with SLNB. The pathologist will evaluate the main variable after the removal of the sentinel lymph nodes in surgery, and will determine if these are affected or not.

The END is the gold standard for detection of metastases. In order to compare the SLNB with this current procedure, the rest of the nodes removed in the END will also be sent to the pathologist. These nodes will be analysed in the standardized way with routine H&E.
This will allow us to calculate the sensitivity, specificity and NPV of the SLNB.

The variable will be measured as a dichotomic qualitative variable:

- Affected lymph nodes:
    - Presence of tumor cells as macrometastasis (>0.2mm) in the lymph nodes removed in the END or in the SLNs detected by routine H&E stain.
    - Presence of tumor cells in the SLN as micrometastasis (<2mm and >0.2mm). Included in this group are the isolated tumor cells (<0.2mm). The detection of micrometastasis will be with H&E at 150µm SSS and with the lymph nodes that resulted negative we will use IHC with anti-cytokeratin antibodies AE-1/AE-3 to detect isolated tumor cells. (18) (42)

- Non-affected lymph nodes: No presence of tumoral cells are found in the SLNs or in the rest of the nodes removed in the END.

Secondary outcome variable:

- The number of lymph nodes affected will be analysed as a quantitative variable and stratified in:
  - Macrometastasis; detected either in SLNs or in the nodes removed in the END.
  - Micrometastasis or isolated tumor cells detected in SLNs.

- Lymph nodes levels: to evaluate in which neck levels the SLNs are found we will use a qualitative variable dividing the levels of the neck using the surgery consensus by the AAO-HNS (Annex 1).
  - Level I(including IA and IB)
  - Level II (including IIA and IIB)
  - Level III
  - Level IV
  - Level V (including VA and VB)
  - Level VI
  - Retropharyngeal space

- Side of neck with metastasis detected by the SLNB: A qualitative variable will be used to differentiate in which side of the neck the SLNs are located with respect to the primary tumor.
  - Ipsilateral: found on the same side of the neck as the primary tumor.
  - Contralateral: found on the opposite side of the neck compared with the primary tumor.
  - Bilateral: found on both sides of the neck.

Covariables:

- HPV (p16 protein)

  In all participants will be using IHC identification in the primary tumor to detect over-expression of p16 protein. The p16protein is the most common genotype of all HPV DNA-positive cancers, with more than 87% of HPV-associated oropharyngeal cancers.

  The variable will be measured as a dichotomic qualitative variable:

  - HPV-positive: In the IHQ, a p16 protein cytoplasmatic expression is shown over 70 per cent of the malignant cells
  - HPV-negative: There is no an over-expression of p16 protein detected by IHQ in the malignant cells.

- Primary tumor localization in the oropharynx:
  - Tonsils
  - Tongue base
  - Soft palate
  - Pharyngeal walls (anterior and posterior)

- Tumor size: it will be evaluated as a qualitative variable according to the TNM of oropharynx.
  - T1 (non in situ-2cm)
  - T2 (2cm-4cm)

8.3.2 LONGITUDINAL STUDY

Independent variable

The independent variable is the lymph node involvement detected after the cross-sectional study by the SLNB-END procedures.

The cohort will be divided between two groups in a dichotomic qualitative variable.

- Affected lymph nodes: patients that after the SLNB-END are upstaged from N0 to N+.

- Non-affected lymph nodes: No presence of tumoral cells are found in the SLNs or in the rest of the nodes removed in the END.

Dependent variable

Local control of the disease at 2 years

The first two years after the initial treatment are considered the ones with highest risk of recurrences. Therefore, the control in this period is more strict. Otorhinolaryngologist, radiologist and pathologist will conduct the follow-up.
The frequency of visits is going to be every 16 weeks during the first two years and every six months until the fifth year.

The patients are going to be evaluated clinically by inspection, palpation and with video-endoscopy. If the patients detect a new symptom between these periods of time, additional visits will be performed.

- The clinical evaluation will consist of:
    - Clinical history: The patients will be asked for the main typical symptoms from the oropharynx area (e.g pain, sore throat or tongue, otalgia and difficulty swallowing)
    - Physical examination: Inspection and palpation of the neck in order to check if there are any adenopathies.
    - Flexible video-endoscopy: Visual evaluation of the oropharynx and the surrounding area to detect any lesion.
- US control will be every 3 months during the first 2 years and every 6 months the next 3 years. In case of a suspicious lymph node, the

radiologists can perform a US with fine needle aspiration biopsy to confirm the diagnosis with the pathologist. The parameters of the US to identify the suspicious lymph nodes are going to be:

  - Size: >8-10 mm diameter
  - Hypoechogenicity
  - Round shape: short/long ratio >0.5
  - Absence of echogenic hilum
  - Colour Doppler with peripheral or mixed vascularity

- Image control will be done every 6 months the first two years and once a year the next 3 years. The same image test that was used for the diagnosis will be performed.
- The radiologist will evaluate distant metastasis. Coinciding with the locoregional control, we are going to perform a thoracic CT once a year during the 5 years of follow-up.

After these years follow-up we will evaluate:

- Local recurrence of the primary tumor confirmed by the pathologist.
- Regional nodal recurrence confirmed by the pathologist.
- Metastasic disease.

<u>The survival rate at 5 years</u>

- Overall survival rate: The percentage of patients in the study that are still alive five years after the treatment.
- Disease-free survival rate: The percentage of patients in the study free of disease 5 years after the treatment.

Covariables:

- Age: We will define age as a qualitative variable, stratifying it in two groups. HPV positive patients are being diagnosed at younger ages. We believe this age cut will reflect the epidemiological trend, where the percentages of positive HPV are increasing in our environment.
  - >65 years
  - <65 years

- Gender:
  - Male
  - Female

- Primary tumor localization in the oropharynx:
  - Tonsils
  - Tongue base
  - Soft palate
  - Pharyngeal walls (anterior and posterior)

- Tumor size: it will be evaluated as a qualitative variable according to the TNM of oropharynx (Annex 2)
  - T1 (non in situ-2cm)
  - T2 (2cm-4cm)

- Metastasis: We will differentiate, through a qualitative variable, if micrometastasis can affect the prognosis.
  - Macrometastasis (>0.2mm)
  - Micrometastasis (<2mm and >0.2mm). Included in this group are the isolated tumor cells (<0.2mm).

- HPV (p16 protein):

  It's widely know that the HPV has an important impact in the prognosis, HPV-associated patients have a better prognosis, so it's a distinguishing characteristic to take into consideration.

  In all participants will be using IHC identification in the primary tumor to detect over-expression of p16 protein.
  The variable will be measured as a dichotomic qualitative variable:
  - HPV-positive: In the IHQ, a p16 protein cytoplasmatic expression is shown over 70 per cent of the malignant cells
  - HPV-negative: There is no an over-expression of p16 protein detected by IHQ in the malignant cells.

- Tobacco: It will be evaluated with packages/ year index, which indicates the risk by classifying the smokers in a qualitative variable:

$$\frac{\text{(Number of cigarettes /day) x years smoking}}{20}$$

  - Low risk: ≤ 20 packages/year
  - Moderate risk: 21-40 packages/year
  - High risk: ≥41 packages/year

- Post-surgery radiotherapy: We will differentiate the patients that need post-surgery radiotherapy after the removal of the primary tumor or after the neck dissection, from the ones that do not need it. The patients staged pN2+ or pN1 with extracapsular spread will need adjuvant RT.

  - Post-surgery radiotherapy
  - Non post-surgery radiotherapy

- Post-surgery chemotherapy: Adjuvant treatment with chemtherapy will be according the protocols. Patients with extracapsular invasion and/or involvement (<1mm) of the surgical margins around the primary tumor will need chemotherapy. As well as if is decided by the committee.
    - Post-surgery chemotherapy
    - Non post-surgery chemotherapy

8.4 PROCEDURES

Sentinel lymph node biopsy

The SLNB concept states that the sentinel lymph node is the first node to which a tumor will metastasize via the lymphatic system. According to this theory, only one or a few lymph nodes will be affected by tumoral cells before they have spread to the rest of the lymph nodes.
This technique needs a multidisciplinary team; including nuclear medicine, surgery and pathology.

The combination of radiolocalitzation and blue-dye mapping will be used(43)(44)(45).

- Pre surgery:

In the medicine nuclear department, an isotopic lymphoscintigraphy will be made to achive a dynamic mapping of the lymphatic drainage in the tumor region. The tumor outline area is injected whit a radiolabeled nanocolloid (Nanocoll TC99). To avoid imprecise injections and for the comfort of the patient, local anaesthesia will be given. The first dynamic scan sequence starts immediately after the injection, and it is followed by an extended sequence until 20-30min passes. At the end of dynamic acquisition, 5 minutes anterior, and both sides lateral static images will be taken, checking the nodal hyperactivity (“hot spots”). If hot spots are not clearly depicted, static images will be repeated after 30, 60 and 120min.

At the end the, SLNs will be located using a gamma probe and their position will be marked on the skin. This procedure will be done the day before the surgery.

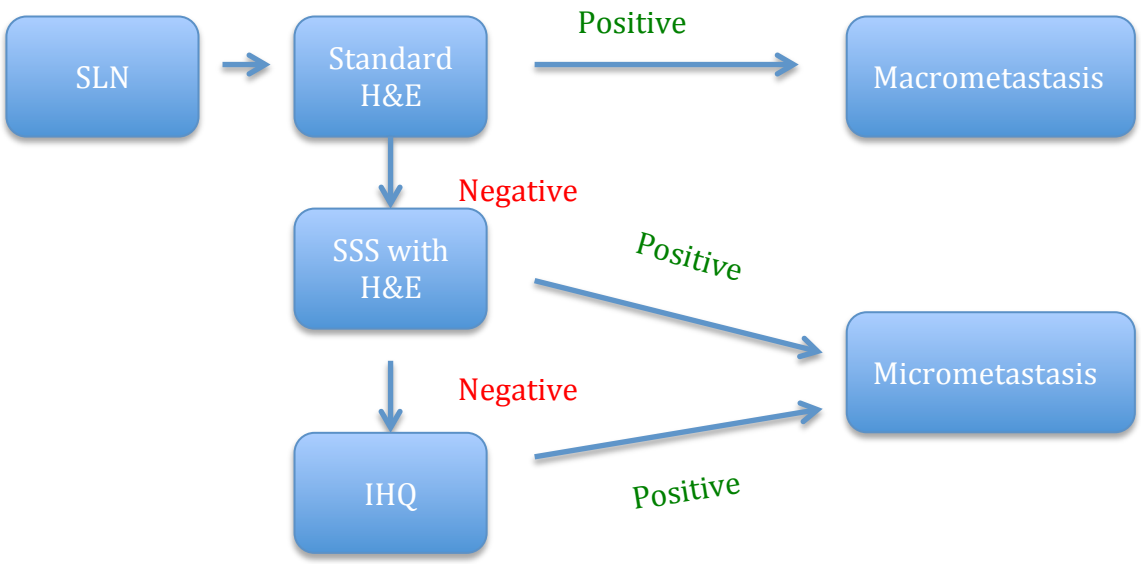
– Surgery:

In the operative room, the day after the SLNs detection, an injection of blue-dye in the points used for the lymphoscintigraphy, will be carried out to improve the detection by allowing a visual help with the staining of the SLNs. After opening the neck, the gamma probe is used for guidance, together with the visual help of the blue-dye, to detect de lymphatic sentinel nodes an proceed to the dissection and excision of them.

– Pathologic evaluation: (42)(18)(46)

The SLN will be fixed in formalin and embedded in paraffin.

First, the nodes will be trimmed in sections of maxium 2.5mm thick and will be examined with standard H&E staining for the detection of macrometastasis. The ones that result negative will be evaluated with H&E at 150μm step serial sections (SSS) for the detection of micrometastasis. Finally the nodes that remain negative will be evaluated with immunohistochemistry (IHC), anti-cytokeratin antibodies AE-1/AE-3, allowing the detection of isolated tumor cells. Pathologic evaluation of the rest lymph nodes from the END will be analysed with the standardized method with H&E only.



Pathologic analysis for the SLNs

8.5. METHODS OF DATA COLLECTION

For data collection, the surgeons of the Head and Neck Unit will be informed about the study that is being carried out, as well as the nuclear medicine department, the pathology department and the radiology department.

Each recruited patient has to sign the consent form after being informed about the study (Annex 3 and Annex 4). Most data will be collected from the clinical history of the participating patients and it will be recorded in a database.
We will create a method to anonymize the participants.
In order to prevent researchers from being able to identify the results of the SLNB we will codify them.

8.5.1 CROSS-SECTIONAL STUDY

The corresponding information will be obtained from each department:

- The nuclear medicine department will report about the lymphoscintigraphy and SLNs localization and will also assist in its verification after the surgeon removes them. This information will be used to identify in which level and side of the neck the SLN is found.
- First, following the protocoled management, the surgeon will remove the primary tumor. Next, the surgeon will detect the SLNs and report about the localization, together with blue-dye support previously injected. Once the SLNs are removed, the surgery will continue to complete the SND.
- One pathologist will report about the presence or absence of tumor cells in the SLNs according to whether they are macrometastasis, micrometastasis or isolated tumor cells. Furthermore, another pathologist will analyse the rest of the nodes from the SND with the routine procedure.

<table>
<tr><th></th><th>Day before Surgery</th><th colspan="2">Day of the Surgery</th><th>Post Surgery</th></tr>
<tr><td rowspan="2">Nuclear Medicine Department</td><td rowspan="2">Radiolabeled nanocollid (Tc99) injection and lymphoscintigraphy

Localization by handed gamma probe of the SLNs and marking at the position on neck skin</td><td>Before surgery</td><td>During surgery</td><td rowspan="2"></td></tr>
<tr><td></td><td>SLNs detection by handed gamma probe</td></tr>
<tr><td>Surgeon</td><td></td><td>Blue-dye injection</td><td>SLNs removal and END</td><td></td></tr>
<tr><td>Pathologist</td><td></td><td colspan="2"></td><td>SLNs and END nodes pathologic analysis</td></tr>
</table>

8.5.2 LONGITUDINAL STUDY

After the cross-sectional study and the adjuvant treatment (if necessary), the patients will be included in the longitudinal study, constituting the consecutive sample.

For the first two years, visits are going to be every 16 weeks, and every six months until the fifth year. The US control will be every 3 months the first two years and every 6 months the next years

To avoid operator dependency two specialists will conduct each visit. In case of different opinions, the two specialists must reach a consensus opinion:

- Two otorhinolaryngologist in charge of the clinical history and the physical exploration.
- Two expert radiologists will report about US and image findings
- In order to achieve the diagnosis, two pathologists will intervene to report in case of abnormal findings, such as a puncture of a suspicious lymph node or a biopsy of an uncertain lesion.

The data obtained from each visit and each specialist will be recorded in the database to stipulate the recurrences after two years of follow-up.

In the event that a patient does not perform properly the follow-up will be located by phone call. The patients who do not make any visits during those 5 years will be classified as losses.

In addition, to detect deaths during the follow-up, we will use the hospital register or the “Indice Nacional de Defunciones” to find deceased patients.

9. STATISTICAL ANALYSIS

9.1 CROSS-SECTIONAL STUDY

9.1.1 Univariate analysis

The variables in this study, both the main and most of the secondary ones, and the covariables are defined as qualitative or categorical variables. Consequently, the results will be expressed as percentages.

The number of sentinel lymph nodes will be considered a qualitative variable and will be expressed as medians and as interquartile ranges (IQR).

9.1.2 Bivariate analysis

We want to evaluate the validity of the diagnostic test based on the sensitivity, the specificity and the negative predict value (NPV).

The main outcome variable, as a dichotomic qualitative variable (affected lymph nodes and non-affected lymph nodes), will allow us to build a table and classify the data in four categories: true positives, false positives, true negatives and false negatives. With these four categories we will calculate the sensitivity, specificity and NPV.

In that table, we will find the results of the SLNB (in the rows) and the results of the gold standard test, which is the END (in the columns).

Sentinel lymph node biopsy	**Elective neck dissection**	
	Lymph node metastasis	**Non lymph node metastasis**
Affected	True Positive (TP)	False Positive (FP)
Non-affected	False Negative (FN)	True Negative (TN)

The results will be stratified by the covariables, with the aim to detect any interaction that might modify the results of the diagnostic test.

Sensitivity= TP/(TP+FN)

Specificity= TN/(TN+FP)

Since the sample is not random we will use the Bayes theorem to estimate the NPV.

9.2 LONGITUDINAL STUDY

9.2.1 Descriptive analysis

Qualitative variables will be summarized by proportions, stratifying between affected and non-affected.

The quantitative variables will be described as medians and as interquartile ranges (IQR).

We will estimate with Kaplan-Meier and we will represent the curves of survival stratifying between the two groups, affected and non-affected.

9.2.2 Bivariate inference

We will answer the differences in the proportions of the qualitative variables obtained between the two groups (affected and non-affected) through the Chi-squared and the exact Fisher test.

We will also answer the differences in the medians of the two groups of study with the Mann-Whitney U test.

And the differences between the survivals curves obtained will be analysed through the long-rank test.

9.2.3 Multivariate analyses

We will assess the recurrences at 2 years and the survival at 5 years by a Cox regression on the explanatory variable of interest, which is the lymph node involvement, and it will be adjusted by the covariables.
We consider a statistically significant difference the “p value” under 0.05.

10. ETHICAL ASPECTS

The SLNB is an invasive procedure that implies an injection of the radioactive colloid. Although this procedure is not included in the current guidelines, it has a very low morbidity. Moreover, the patients that have any SLNB contraindications are excluded of the study so we consider that the right of non-maleficence is not affected.

The CEIC (Comissio d'Ètica d'Investigació Clínica) of the Hospital Universitari Girona Dr. Josep Trueta will evaluate the protocol for ethics approval. In addition, their recommendations will be taken into consideration.

This study will be conducted in accordance with the ethical considerations announce in the *Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects* by the World Medical Association (revised in 2013).

The study will also be in accordance with the Spanish Law 14/2007 3 de Julio, de Investigación Biomédica.

The participants included in the study will be informed and asked to sign the informed consent before entering the study. All the participants information will be confidential and anonymous according to Law 15/1999, 13 de Diciembre, de Protección de Datos Personales.

All investigators will have to declare no conflict of interests.

11. LIMITATIONS OF THE STUDY

- SLNB technique is an operator-dependent procedure that requires trained nuclear medicine physicians, otorhinolaryngologist and pathologist. Although the specialists are well trained, this procedure requires practice. In order to control the dependence of the operator on this procedure, training will be carried out for the specialists in charge of it.

  Because of the variability of expertise in each institution, the external validity of the study can be affected. A further multi-centric study would be required to confirm our results.

- There is the possibility that a confounding bias occurs if some unknown covariables are not included in the study.

- The sample will be selected by a non-probabilistic method with strict inclusion and exclusion criteria; this could originate a lack of representativeness of the population.

- Information bias will be avoided by performing the visits by more than one specialist and demanding a unified conclusion will avoid the interobserver variability that might originate. Two different pathologists will conduct the pathologic analysis of the SLNB and the END.

  The physicians that conduct the follow-up of the patients won't know the result of the SLNB.

- During the follow-up we can have some loses, although we expect them be a very low percentage, because the visits programmed are included in the usual following of these oncologic patients.

- The investigators will have to declare no conflict of interest.

12. WORK PLAN AND TIME SCHEDULE

Phase 1: Coordination (4 months)

- Protocol design (3 months): During this period the principal investigators will perform a draft of the initial protocol through a bibliographic research.
- First informative meeting: The protocol will be presented to the research team that will take part of the investigation. We will ensure that all researchers agree with the protocol and any suggestions will be taken into consideration.
- Ethical Committee evaluation and approval: We will present the protocol to the CEIC for its ethics approval before starting the study.
- Coordination meeting: We will organise a meeting between all the researchers, to set up the execution plan and to be sure that all the people involved understand their roles in the procedures and the recollection of data.

Phase 2: Field work and data collection (6.5 years)

- Sampling recruitment and SLNB intervention: At least, 19 months will be required to select 32 patients that fulfil the inclusion and exclusion criteria. Patients will undergo surgery of the primary tumor and SLNB- END procedures. The recruitment will stop when the necessary sample is completed.
- Patients' evaluation: After the SLNB-END (+/- adjuvant treatment) and according to its results, we will include each patient anonymously in one of the groups of the independent variable. The follow-up period will start. This period will last 5 years.
- Data collection: All the data recollected from the SLNB-END and from the follow-up will be entered in a database. Every investigator will be responsible to collect the data correctly following the anonymity system.
- Control meetings: During the period of the study several meetings will be organised with the researchers to resolve possible problems and to ensure that the data collection is correctly done.

Phase 3: Data analysis (8 months)

- Statistical analysis: A first preliminary data analysis will be performed after the cross-sectional test, in order to be able to create the two groups necessary for the follow-up. Afterwards, once all data is collected the statistician will perform the final statistical analysis of the diagnostic test and the follow-up.
- Interpretation of the results: The research team will meet with the statisticians to interpret the results and formulate the conclusions.
- Final report elaboration

Phase 4: Publication and scientific dissemination of the results (2 months)

- Publication of the results: The results will be presented to specific magazines and scientific journals for its publication.
- National Congress presentation: We will present the results of our study in the annual conferences on this topic.

12.1 TIME SCHEDULE

	2018		2019			2020		2021	2022	2023	2024	2025		2026		
	N	D	J	F	M - D	J - S	O - D					J-S	O-D	J-F	M-A	M-J
PHASE 1																
Protocol design																
First informative meeting																
CEIC approval																
Coordination meeting																
PHASE 2																
Sample recruitment and SLNB																
Patient's evaluation																
Data collection (follow-up)																
Control meetings																
PHASE 3																
Statistical analysis																
Interpretation of the results																
Final report elaboration																
PHASE 4																
Publication																
Congress presentation																

13. BUDGET

	Cost	Unit/Hours	Total Cost
Staff			
Statistician	35€/hour	40h	1.400€
Materials			
Tc99 radiocolloid / Lymphoscintigraphy	650€/Unit	32 units	20.800€
Blue-Dye	10€/Unit	32 units	320€
Anti-cytokeratin antibodies AE-1/AE-3	175€/Unit	50 units	8.750€
Others: literature, information and consent documents printing.	100€		100€
Publication			
Publishing fees	1500€		1.500€
Diffusion costs			
Inscription to Conferences and congresses	300€/person	x2 researchers	600€
Transport, accommodation and other expenses	400€/person	x2 researchers	800€
		TOTAL COST:	34.270€

The investigation group will contribute in their daily routine as part of their work in the hospital so they won't receive any financial compensation.

We calculate that we can find more than one sentinel node per neck that will need an IHQ analysis, but on the other hand in some of them it will only be necessary to use H&E for its detection, which is already part of the standardized protocol.

The follow-up of the patients after the treatment will be part of the normal follow-up carried out in the hospital, therefore it won't suppose any extra cost to the study

14. CLINICAL AND HEALTHCARE IMPACT

The management of the N0 patients in head and neck cancers continues to be controversial, but over the years we have seen a progress towards a more conservative management. Radical neck dissection, which was the elected procedure for all necks in the beginning, gave way to the selective neck dissection in those patients in which lymph node metastasis cannot be found.

The SLNB continues with this tendency towards a more conservative management of the neck. It has shown very promising results in the oropharyngeal cancer. Even in the oral cancer some guidelines propose this technique in the diagnostic algorithm.

With this study we want to demonstrate that the SLNB technique has a high sensitivity, specificity and NPV in the Hospital Universitari Dr.Josep Trueta. This would mean that this technique is valid to diagnose the cervical lymph node metastases in this institution and other institutions would probably achieve similar results.

Furthermore, if our hypothesis is confirmed, we will be able to perform further studies to see if we can avoid the END in patients in which SLNB resulted negative and only perform a treatment of the neck if the SLNB shows a positive result. This would imply an immediate benefit for the patients as well as, in our opinion, a reduction of the costs, as a big number of patients would not be submitted under surgery of the neck because of a negative result in the SLNB.
Another piece of valuable information that we would achieve with this technique is the knowledge of the cervical lymphatic drainage of each tumor.
In patients with a positive SLNB, we will be able to see in which side an in what level of the neck the SLN has been found, and therefore perform a neck dissection in accordance with this results.

15. BIBLIOGRAPHY

1. McGuire S. World Cancer Report 2014. Geneva, Switzerland: World Health Organization, International Agency for Research on Cancer, WHO Press, 2015. Adv Nutr An Int Rev J. 2016;7(2):418–9.
2. Diz P, Meleti M, Diniz-Freitas M, Vescovi P, Warnakulasuriya S, Johnson NW, et al. Oral and pharyngeal cancer in Europe. Transl Res Oral Oncol. 2017;2:2057178X1770151.
3. Pytynia KB, Dahlstrom KR, Sturgis EM. Epidemiology of HPV-associated oropharyngeal cancer. Oral Oncol. 2014 May 1;50(5):380–6.
4. Winn DM, Lee YC, Hashibe M, Boffetta P, Agudo A, Ahrens W, et al. The INHANCE consortium: Toward a better understanding of the causes and mechanisms of head and neck cancer. Oral Dis. 2015;21(6):685–93.
5. Elrefaey S, Massaro MA, Chiocca S, Chiesa F, Ansarin M. HPV in oropharyngeal cancer: the basics to know in clinical practice. Acta Otorhinolaryngol Ital. 2014 Oct;34(5):299–309.
6. Mehanna H, Evans M, Beasley M, Chatterjee S, Dilkes M, Homer J, et al. Oropharyngeal cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;90–6.
7. Tantiwongkosi B, Yu F, Kanard A, Miller FR. Role of (18)F-FDG PET/CT in pre and post treatment evaluation in head and neck carcinoma. World J Radiol. 2014 May 28;6(5):177–91.
8. Lydiatt WM, Patel SG, O'Sullivan B, Brandwein MS, Ridge JA, Migliacci JC, et al. Head and neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017 Mar;67(2):122–37.
9. Docampo LCI, Arrazubi Arrula • V, Rotllan • N Baste, Carral Maseda • A, Cirauqui • B Cirauqui, Escobar • Y, et al. CLINICAL GUIDES IN ONCOLOGY SEOM clinical guidelines for the treatment of head and neck cancer (2017). Clin Transl Oncol. 2094;20.
10. Argiris A, Karamouzis M V, Raben D, Ferris RL. Head and neck cancer. Lancet (London, England). 2008 May 17;371(9625):1695–709.

11. SNOW GB, ANNYAS AA, SLOOTEN EAV, BARTELINK H, HART AAM. Prognostic factors of neck node metastasis. Clin Otolaryngol Allied Sci. 1982;7(3):185–92.
12. Haberal I, Çelik H, Göçmen H, Akmansu H, Yörük M, Özeri C. Which is important in the evaluation of metastatic lymph nodes in head and neck cancer: Palpation, ultrasonography, or computed tomography? Otolaryngol - Head Neck Surg. 2004;130(2):197–201.
13. Curtin HD, Ishwaran H, Mancuso AA, Dalley RW, Caudry DJ, McNeil BJ. Comparison of CT and MR imaging in staging of neck metastases. Radiology. 1998;207(1):123–30.
14. de Bondt RBJ, Nelemans PJ, Hofman PAM, Casselman JW, Kremer B, van Engelshoven JMA, et al. Detection of lymph node metastases in head and neck cancer: A meta-analysis comparing US, USgFNAC, CT and MR imaging. Eur J Radiol. 2007;64(2):266–72.
15. Ahuja AT, Ying M, Ho SY, Antonio G, Lee YP, King AD, et al. Ultrasound of malignant cervical lymph nodes. Cancer Imaging. 2008 Mar 25;8(1):48–56.
16. Psychogios G, Mantsopoulos K, Bohr C, Koch M, Zenk J, Iro H. Incidence of occult cervical metastasis in head and neck carcinomas: Development over time. J Surg Oncol. 2013;107(4):384–7.
17. Helliwell T, Woolgar J. Standards and datasets for reporting cancers Dataset for histopathology reporting of nodal excisions and neck dissection specimens associated with head and neck carcinomas. 2013
18. Woolgar J, Triantafyllou A. Neck dissections: A practical guide for the reporting histopathologist. Curr Diagnostic Pathol. 2007;13:499–511.
19. Robbins KT, Shaha AR, Medina JE, Califano JA, Wolf GT, Ferlito A, et al. Consensus Statement on the Classification and Terminology of Neck Dissection. Arch Otolaryngol Neck Surg. 2008 May 1;134(5):536.
20. Robbins KT, Clayman G, Levine PA, Medina J, Sessions R, Shaha A, et al. Neck Dissection Classification Update. Arch Otolaryngol Neck Surg. 2002 Jul 1;128(7):751.
21. Ballantyne AJ. Significance of retropharyngeal nodes in cancer of the head and neck. Am J Surg. 1964;108(4):500–4.

22. Gunn GB, Debnam JM, Fuller CD, Morrison WH, Frank SJ, Beadle BM, et al. The impact of radiographic retropharyngeal adenopathy in oropharyngeal cancer. Cancer. 2013 Sep 1;119(17):3162–9.
23. Coskun H, Ferlito A, Medina J, Robbins K, Rodrigo J et. al. Retropharyngeal lymph node metastases in head and neck malignancies. Head Neck. 2011;33(10):1520–9.
24. Chung EJ, Kim GW, Cho BK, Cho SJ, Yoon DY, Rho YS. Retropharyngeal Lymph Node Metastasis in 54 Patients with Oropharyngeal Squamous Cell Carcinoma Who Underwent Surgery-Based Treatment. Ann Surg Oncol. 2015;22(9):3049–54.
25. Paleri V, Urbano TG, Mehanna H, Repanos C, Lancaster J, Roques T, et al. Management of neck metastases in head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016 May;130(S2):S161–9.
26. Sparano A, Weinstein G, Chalian A, Yodul M, Weber R. Multivariate predictors of occult neck metastasis in early oral tongue cancer. Otolaryngol - Head Neck Surg. 2004;131(4):472–6.
27. Imai T, Satoh I, Matsumoto K, Asada Y, Yamazaki T, Morita S, et al. Retrospective observational study of occult cervical lymph-node metastasis in T1N0 tongue cancer. Jpn J Clin Oncol. 2017;47(2):130–6.
28. D'Cruz AK, Vaish R, Kapre N, Dandekar M, Gupta S, Hawaldar R, et al. Elective versus Therapeutic Neck Dissection in Node-Negative Oral Cancer. N Engl J Med. 2015 Aug 6;373(6):521–9.
29. Inoue H, Nibu K, Saito M, Otsuki N, Ishida H, Onitsuka T, et al. Quality of Life After Neck Dissection. Arch Otolaryngol Neck Surg. 2006 Jun 1;132(6):662.
30. Olzowy B, Tsalemchuk Y, Schotten K-J, Reichel O, Harré U. FREQUENCY OF BILATERAL CERVICAL METASTASES IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: A RETROSPECTIVE ANALYSIS OF 352 CASES AFTER BILATERAL NECK DISSECTION. 2010;33:239–43.
31. Schmitz S, Machiels J, Weynand B, Gregoire V HM. Results of selective neck dissection in the primary management of head and neck squamous cell carcinoma. Eur Arch Oto-Rhino-Laryngology. 2009;266(3):437–43.

32. Simo R, Homer J, Clarke P, Mackenzie K, Paleri V, Pracy P, et al. Follow-up after treatment for head and neck cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016 May ;130(S2):S208–11.
33. van den Brekel MWM, Castelijns JA, Reitsma LC, Leemans CR, van der Waal I, Snow GB. Outcome of Observing the N0 Neck Using Ultrasonographic-Guided Cytology for Follow-up. Arch Otolaryngol Neck Surg [Internet]. 1999 Feb 1;125(2):153.
34. Alex JC, Krag DN. The gamma-probe-guided resection of radiolabeled primary lymph nodes. Surg Oncol Clin N Am. 1996 Jan;5(1):33–41.
35. Shoaib T, Soutar DS, Prosser JE, Dunaway DJ, Gray HW, McCurrach GM, et al. A suggested method for sentinel node biopsy in squamous cell carcinoma of the head and neck. Head Neck. 1999 Dec;21(8):728–33.
36. Alkureishi LWT, Burak Z, Alvarez JA, Ballinger J, Bilde A, Britten AJ, et al. Joint practice guidelines for radionuclide lymphoscintigraphy for sentinel node localization in oral/oropharyngeal squamous cell carcinoma. Ann Surg Oncol [Internet]. 2009 Nov;16(11):3190–210.
37. Uren RF, Howman-Giles RB, Chung D, Thompson JF. Role of lymphoscintigraphy for selective sentinel lymphadenectomy. Cancer Treat Res. 2005;127:15–38.
38. Helliwell TR, Giles TE. Pathological aspects of the assessment of head and neck cancers: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016 May;130(S2):S59–65.
39. Cho J-H, Lee Y-S, Sun D-I, Kim M-S, Cho K-J, Nam I-C, et al. Prognostic impact of lymph node micrometastasis in oral and oropharyngeal squamous cell carcinomas. Head Neck. 2016 Apr 1;38(S1):E1777–82.
40. Thompson CF, St. John MA, Lawson G, Grogan T, Elashoff D, Mendelsohn AH. Diagnostic value of sentinel lymph node biopsy in head and neck cancer: A meta-analysis. Eur Arch Oto-Rhino-Laryngology. 2013;270(7):2115–22.
41. Alkureishi LWT, Ross GL, Shoaib T, Soutar DS, Robertson AG, Thompson R, et al. Sentinel node biopsy in head and neck squamous cell cancer: 5-year follow-up of a european multicenter trial. Ann Surg Oncol. 2010;17(9):2459–64.

42. Trivedi N, Ravindran H, Sundram S, Iyer S, Kekatpure V et. al. Pathologic evaluation of sentinel lymph nodes in oral squamous cell carcinoma. Head Neck. 2010;32(11):1437–43.
43. Liu M, Wang SJ, Yang X, Peng H. Diagnostic Efficacy of Sentinel Lymph Node Biopsy in Early Oral Squamous Cell Carcinoma: A Meta-Analysis of 66 Studies. Han G, editor. PLoS One. 2017 Jan 20;12(1):e0170322.
44. Garrel R, Poissonnet G, Temam S, Dolivet G, Fakhry N, de Raucourt D. Review of sentinel node procedure in cN0 head and neck squamous cell carcinomas. Guidelines from the French evaluation cooperative subgroup of GETTEC. Eur Ann Otorhinolaryngol Head Neck Dis. 2017;134(2):89–93.
45. Ross GL, Shoaib T, Soutar DS, MacDonald DG, Camilleri IG, Bessent RG, et al. The first International Conference on sentinel node biopsy in mucosal head and neck cancer and adoption of a multicenter trial protocol. Ann Surg Oncol. 2002;9(4):406–10.
46. Čelakovský P, Kalfeřt D, Smatanová K, Chrobok V, Laco J. Detection of Cervical Lymph Node Micrometastases in Patients with Squamous Cell Carcinoma of the Oral Cavity, Pharynx and Larynx. Acta medica (Hradec Králové) / Univ Carolina, Fac Medica Hradec Králové. 2015;58(2):62–5.
47. Malloy KM. AJCC 8 th Edition Major Updates in Cancer Staging: Implications for OPC treatment?. 2017.

ANNEX 1 - Anatomical division of the lymph nodes by levels

- Sublevel IA (Submental): Lymph nodes within the triangular boundary of anterior belly of the digastric muscles and the hyoid bone.
- Sublevel IB (Submandibular): Lymph nodes within the boundaries of anterior belly of the digastric muscle, the stylohyoid muscle, and the body of the mandible.
- Sublevel II(Upper jugular): Lymph nodes located around the upper third of internal jugular vein and adjacent spinal accessory nerve extending from the level of skull base (above) to the level of the inferior border of the hyoid bone (below). The anterior (medial) boundary is the stylohyoid muscle and the posterior (lateral) boundary is the posterior border of the sternocleidomastoid muscle.
    - Sublevel IIA: Located anterior (medial) to the vertical plane defined by the spinal accessory nerve.
    - Sublevel IB: Located posterior (lateral) to the vertical plane definded by the spinal accessory nerve.
- Level III (Middle jugular): Lymph nodes located around the middle third of the internal jugular vein extending from the inferior border of the hyoid bone (above) to the inferior border of the cricoid cartilage (below). The anterior (medial) boundary is the lateral border of the sternohyoid muscle, and the posterior (lateral) boundary is the posterior of the sternocleidomastoid muscle.
- Level IV (Lower jugular): Lymph nodes located around the lower third of the internal jugular vein extending from the inferior border of the cricoid cartilage (above) tot the clavicle below. The anterior (medial) boundary is the lateral border of the sternohyoid muscle and the posterior (lateral) boundary is the posterior border of the sternocleidomastoid muscle.
- Level V (Posterior triangle group): The superior boundary is the apex formed by convergence of the sternocleidomastoid and trapezius muscle, the inferior boundary is the clavicle, the anterior (medial) is the posterior border of the sternocleidomastoid muscle and the posterior (lateral) is the anterior border of the trapezius muscle.

- Sublevel VA: Located superior from the horizontal plane defined by the lower border of the cricoid cartilage.
- Sublevel VB: Located inferior from the horizontal plane defined by the lower border of the cricoid cartilage.

– Level VI (Anterior compartment group): Lymph nodes in this compartment include the pretracheal and paratracheal node, precricoid node and the perithyroidal nodes including the ones along the recurrent laryngeal nerves. The superior boundary is the hyoid bone, the inferior is the suprasternal notch, and the lateral boundaries are the common carotid arteries.

Table 3. Anatomical Structures Defining the Boundaries of the Neck Levels and Sublevels

	Boundary			
Level	Superior	Inferior	Anterior (Medial)	Posterior (Lateral)
IA	Symphysis of mandible	Body of hyoid	Anterior belly of contralateral digastric muscle	Anterior belly of ipsilateral digastric muscle
IB	Body of mandible	Posterior belly of muscle	Anterior belly of digastric muscle	Stylohyoid muscle
IIA	Skull base	Horizontal plane defined by the inferior body of the hyoid bone	Stylohyoid muscle	Vertical plane defined by the spinal accessory nerve
IIB	Skull base	Horizontal plane defined by the inferior body of the hyoid bone	Vertical plane defined by the spinal accessory nerve	Lateral border of the sternocleidomastoid muscle
III	Horizontal plane defined by inferior body of hyoid	Horizontal plane defined by the inferior border of the cricoid cartilage	Lateral border of the sternohyoid muscle	Lateral border of the sternocleidomastoid or sensory branches of cervical plexus
IV	Horizontal plane defined by the inferior border of the cricoid cartilage	Clavicle	Lateral border of the sternohyoid muscle	Lateral border of the sternocleidomastoid or sensory branches of cervical plexus
VA	Apex of the convergence of the sternocleidomastoid and trapezius muscles	Horizontal plane defined by the lower border of the cricoid cartilage	Posterior border of the sternocleidomastoid muscle or sensory branches of cervical plexus	Anterior border of the trapezius muscle
VB	Horizontal plane defined by the lower border of the cricoid cartilage	Clavicle	Posterior border of the sternocleidomastoid muscle or sensory branches of cervical plexus	Anterior border of the trapezius muscle
VI	Hyoid bone	Suprasternal	Common carotid artery	Common carotid artery

Anatomical structures defining the Boundaries of the neck levels and sublevels from Robbins et al. (19)

ANNEX 2 - TNM of the oropharyngeal carcinoma 8th Edition Staging Manual (47)

T CATEGORY	T CRITERIA
T0	No primary identified
T1	Tumor 2 cm or smaller in greatest dimension
T2	Tumor larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumor larger than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Moderately advanced local disease; tumor invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate, or mandible or beyond[b]

T Category for HPV (p16-Positive) Oropharyngeal Cancer from 8th Edition Staging Manual.

T CATEGORY	T CRITERIA
Tx	Primary tumor cannot be assessed
Tis	Carcinoma in situ
T1	Tumor 2 cm or smaller in greatest dimension
T2	Tumor larger than 2 cm but not larger than 4 cm in greatest dimension
T3	Tumor larger than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Moderately advanced or very advanced local disease
T4a	Moderately advanced local disease; tumor invades the larynx, extrinsic muscle of tongue, medial pterygoid, hard palate, or mandible[b]
T4b	Very advanced local disease; tumor invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base or encases carotid artery

T Category for non- HPV (p16-Negative) Oropharyngeal Cancer from 8th Edition Staging Manual.

N CATEGORY	N CRITERIA
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	One or more ipsilateral lymph nodes, none larger than 6 cm
N2	Contralateral or bilateral lymph nodes, none larger than 6 cm
N3	Lymph node(s) larger than 6 cm

N Category for HPV (p16-Positive) Oropharyngeal Cancer from 8th Edition Staging Manual.

N CATEGORY	N CRITERIA
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or smaller in greatest dimension and ENE-negative
N2	Metastasis in a single ipsilateral lymph node larger than 3 cm but not larger than 6 cm in greatest dimension and ENE-negative; or metastases in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and ENE-negative; or metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and ENE-negative
N2a	Metastasis in a single ipsilateral lymph node larger than 3 cm but not larger than 6 cm in greatest dimension and ENE-negative
N2b	Metastasis in multiple ipsilateral lymph nodes, none larger than 6 cm in greatest dimension and ENE-negative
N2c	Metastasis in bilateral or contralateral lymph nodes, none larger than 6 cm in greatest dimension and ENE-negative
N3	Metastasis in a lymph node larger than 6 cm in greatest dimension and ENE-negative; or metastasis in any lymph node(s) and clinically overt ENE-positive
N3a	Metastasis in a lymph node larger than 6 cm in greatest dimension and ENE-negative
N3b	Metastasis in any node(s) and clinically overt ENE-positive

N Category for non-HPV associated (p16-Negative) Oropharyngeal Cancer from 8th Edition Staging Manual.

Annex 3 – Information sheet

HOJA DE INFORMACIÓN

Encabezado.

1. Título de proyecto: Validation of Sentinel lymph node biopsy in early stages of oropharyngeal cancer
2. Centro, unidad, servicio: Hospital Universitari Dr.Josep Trueta de Girona, Unidad Oncológica de Cabeza y Cuello, servicio de Otorrinlolaringología

Datos de la Investigación:

1. Descripción general:

Considerando la enfermedad o proceso que usted padece, le solicitamos su consentimiento para participar en un estudio del que le informamos a continuación. Antes de decidir si quiere participar o no, le rogamos lea detenidamente este documento que incluye la información sobre este proyecto. Puede formular todas las preguntas que le surjan y solicitar cualquier aclaración sobre cualquier aspecto del mismo.

El lugar donde se procesará la muestra será en el laboratorio clínico del Hospital Universitari Dr.Josep Trueta de Girona.
El proyecto cuenta con el informe favorable del Comité Ético de Investigación Clínica del Hospital universitario Dr.Josep Trueta de Girona.

Es probable que no reciba ningún beneficio personal por su participación en este estudio. En cualquier caso, los datos recogidos en el mismo podrán derivar en un mayor conocimiento de su enfermedad o condición objeto de estudio.

Su participación en este estudio es voluntaria: Si usted decide no participar recibirá todos los cuidados médicos que pudiera necesitar y su relación con el equipo médico que le atiende no se verá afectada.

2. Propósito del estudio:

El principal objetivo de este estudio es evaluar la eficacia del ganglio centinela para detectar y establecer la posible afectación locoregional ganglionar que presentan los pacientes con cáncer de orofaringe.

Los ganglios linfáticos son la principal via de diseminación del cáncer de orofaringe y su afectación conlleva un pero pronóstico para el paciente. Actualmente los pacientes, que por clínica o por pruebas de imagen, no es posible detectar una afectación ganglionar locoregional son valorados para ofrecerles un tratamiento quirúrgico por el vaciamiento de los mismos para su posterior análisis. En algunos casos, una vez se a procedido a su análisis, no se encuentran ganglios afectados. Con la técnica del ganglio centinela queremos detectar estos pacientes que no son necesarios someterse a un cirugía para realizar el vaciamiento ganglionar.

3. Procedimientos del estudio:

Usted seguirá el procedimiento terapéutico rutinario, pero se le realizara un procedimiento diagnostico adicional. El procedimiento del ganglio centinela se realizara en dos partes, un dia antes de la cirugía y la siguiente durante la cirugía.

El día antes se le realizará una inyección de Tc99 en la zona del tumor seguido de una limfoescintografia, una prueba de imagen para detectar donde se sitúan los ganglios centinela y así poder marcarlos. El día de la cirugía se le inyectará un tinción con azul de metileno en la zona del tumor para ayudar a la detección del ganglio centinela durante la cirugía junto con una gamma sonda serán localizaos y posteriormente extirpados. A continuación se realizara el vaciamiento ganglionar habitual. Los ganglios centinela extraídos junto con los ganglios extraídos en el vaciamiento cervical serán estudiados histológicamente.

Una vez haya completado su tratamiento realizará un seguimiento protocolizado que coincidirá con el seguimiento habitual, En él deberá acudir a una serie de visitas de control cada 4-8 semanas durante los primeros dos años y posteriormente cada 3-6 meses hasta los cinco años. En estas visitas de control se realizará una evaluación clínica de los síntomas y una exploración física junto con una endoscopia directa de las vías superiores. En función de los hallazgos en las visitas sucesivas podrían ser necesarias pruebas adicionales.

4. Muestras a recoger:

Como parte de este proyecto aprobado por el Comité Ético de Investigación Clínica del Hospital Universitari Dr.Josep Trueta de Girona se le va a extraer una muestra para utilizarla con fines de investigación, con objeto de aumentar los conocimientos sobre la patología o proceso objeto de estudio, y desarrollar nuevas estrategias y terapias aplicables a futuros pacientes.

La extirpación de tejido se realiza con fines diagnósticos y terapéuticos, pero frecuentemente no se estudia toda la muestra sino que, tras realizar los estudios histopatológicos de las zonas representativas de la lesión, se suele destruir el resto del tejido.

Su colaboración es gratuita, por lo que renuncia a cualquier derecho de naturaleza económica, patrimonial o potestativa sobre los potenciales beneficios que puedan derivarse de manera directa o indirecta de las investigaciones que se lleven a cabo con las muestras que cede para investigación.

5. Riesgos e inconvenientes para el participante

El principal inconveniente del estudio es el deber de asistir el día anterior a la cirugía para someterse a la inyección del radiotrazaor de Tc99 y la posterior limfoescintografia.
a
Las reacciones adversas al trazador son muy infrecuentes, si usted a padecido alguna reacción alérgica relacionada le aconsejamos no participar en el estudio.

6. Derechos del participante

Usted puede revocar su consentimiento y sus efectos en cualquier momento, incluida la posibilidad de la destrucción o de la anonimización (destrucción del código que vincula la muestra con su identidad) de sus muestras sin necesidad de dar explicaciones y sin ningún perjuicio en su tratamiento médico. En este caso, la revocación no se extenderá a los datos resultantes de las investigaciones que ya se hayan llevado a cabo. Asimismo tiene derecho a incluir las restricciones que desee respecto del uso de sus muestras.

.

Confidencialidad

Toda la información relacionada con el estudio es estrictamente confidencial según la L.O.P.D. 15/1999 de 13 de Diciembre.

Sus derechos de acceso, rectificación, cancelación y oposición puede ejercitarlos ante la Unidad de cabeza y cuello del Hospital Universitari Dr.Josep Trueta de Girona.

Representantes del Comité Ético de Investigación Clínica del Hospital y de las Autoridades Sanitarias Españolas podrán tener acceso a sus registros médicos con el fin de controlar y garantizar la correcta realización del estudio. Los resultados del estudio podrán ser comunicados en reuniones científicas, Congresos Médicos o publicaciones científicas, sin embargo se mantendrá una estricta confidencialidad sobre la identidad de los pacientes.

Annex 4- Informed Consent

CONSENTIMIENTO INFORMADO

Datos del estudio para el que se otorga el consentimiento

Investigador principal
Titulo proyecto
Centro

Datos del participante/paciente
Nombre

_____

Persona que proporciona la información y la hoja de consentimiento
Nombre

_____

1. He leído, he sido informado y comprendo el contenido de la presente hoja de Información, lo que acredito con mi firma en prueba de mi consentimiento en todo lo que en ella se contiene.
SI ☐ NO ☐

2. He preguntado y aclarado las posibles dudas al Dr./Dra……..

3. Entiendo que mi participación es voluntaria y gratuita y comprendo que puedo solicitar la revocación de este consentimiento en cualquier momento, sin tener que ofrecer explicaciones y sin que esto repercuta en mis cuidados médicos presentes y/o futuros.
SI ☐ NO ☐

5. Autorizo, cuando sea preciso, a que se pongan en contacto conmigo para solicitar información adicional o para recibir información relevante para mi salud o la salud de mis familiares derivada de la investigación.
SI ☐ NO ☐

En …….. a …... de ……. de 20……

Fecha: Firma del Participante/paciente

Fecha: Firma del Investigador o persona que proporciona la información