FINAL DEGREE PROJECT

NEW EPIDEMIOLOGIC TRENDS IN HUMAN PAPILLOMAVIRUS-RELATED OROPHARYNX CANCER IN GIRONA: A CROSS-SECTIONAL STUDY

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1. ABBREVIATIONS

CT	Computed tomography
DNA	Deoxynucleic acid
E6	Early oncoprotein 6
E7	Early oncoprotein 7
HPV	Human papillomavirus
ISH	In situ hybridization
MRI	Magnetic resonance imaging
OPSCC	Oropharyngeal squamous cell carcinoma
ORL	Otorhinolaryngology
PCR	Polymerase Chain Reaction
Rb	Retinoblastoma
RT	Radiotherapy
SCC	Squamous cell carcinoma

2. ABSTRACT

BACKGROUND: Oropharyngeal squamous cell carcinoma is a disease on the rise, being this epidemiological trend in relation to infection by human papillomavirus (HPV). HPV is now considered a major etiological factor for this subtype of cancer, which affects a particular kind of patient: young adults, without exposition to alcohol or tobacco, and high risk sexual behavior. Recent studies point in the direction of HPV-related cancer having a much better prognosis, what may translate in the future into de-escalation protocols of treatment, specially designed for this subgroup of patients.

OBJECTIVES: To analyze the prevalence of Human Papillomavirus infection among patients diagnosed in Hospital Universitari Dr. Josep Trueta.

The secondary objectives deal with analyzing the relation between Human Papillomavirus infection and different covariates, in order to provide our doctors with a wider conception of the kind of patients there are treating with.

DESIGN: A cross-sectional study has been designed for this purpose.

SETTING AND PARTICIPANTS: The study will be performed in Hospital Universitari Dr. Josep Trueta, and a non-probabilistic consecutive sampling is going to be performed; therefore, every patient older than 18 years old diagnosed with oropharyngeal squamous cell carcinoma will be asked to participate in it.

OUTCOMES: Our variable of interest, or outcome variable, is the presence of human papillomavirus infection in our population of study, measured by p16 staining via immunochemistry. It will be analyzed as a nominal dichotomous qualitative variable.

KEYWORDS:

- Human Papillomavirus
- Oropharyngeal cancer
- P16
- Prevalence
- Epidemiology

3. INTRODUCTION

3.1 BACKGROUND

OROPHARYNGEAL SQUAMOUS CELL CARCINOMA: NEW TRENDS IN EPIDEMIOLOGY

Head and neck cancer includes tumors that arise from the oral cavity, oropharynx, hypopharynx larynx and sinonasal tract. These tumors share common characteristics, including a male predominant appearance in the 5-6th decade of life, a strong etiological link with prior tobacco, alcohol use or betel nut chewing, and histopathological resemblance (1). 90% of head and neck cancers are squamous cell carcinomas (SCC). The estimated annual burden of head and neck SCC is 650.555 incident cases worldwide (2), of which 85.000 incident cases are located in the oropharynx.

Public health efforts in recent years have resulted in a decrease of smoking rates in developed countries. This has been reflected in a dramatic decrease in the past two decades in the incidence of smoking-related cancers of the oral cavity, pharynx and larynx. It was expected that oropharyngeal squamous cell carcinoma (OPSCC) would decrease in incidence in relation to decreasing smoking rates, but there has been only a plateau in OPSCC incidence followed more recently by a dramatic increase (as shown in *Figure 1*) (3).

It has been observed that this increase in OPSCC incidence includes a number of middle-aged white men that often are nonsmokers and nondrinkers (that is, people who do not have traditional risk factors for OPSCC) (4).

The rising incidence of this particular type of oropharyngeal squamous cell carcinoma, concerning base of the tongue, tonsils, soft palate, uvula, and posterior and lateral pharyngeal wall has also been reported as a discrepancy between developed and developing countries (this pathology is becoming relevant exclusively in developed nations) (2), thus encouraging researches to look for new risk factors contributing to the rising incidence of OPSCC. This search led to human papillomavirus (HPV).

HPV infection has been increasingly recognized as a major etiological factor for a subset of head and neck SCC, including mostly OPSCC. It has become clear that HPV plays a pathogenic role in this subset of head and neck cancers, with distinct epidemiologic, clinical and molecular characteristics (1) that will be reviewed later in this text.

The incidence of HPV-positive oropharyngeal squamous cell carcinoma is increasing markedly, and it is not hyperbole to call this an epidemic. Of the estimated 85.000 cases of oropharyngeal cancer occurred worldwide in 2008, at least 22.000 were HPV-positive (5).

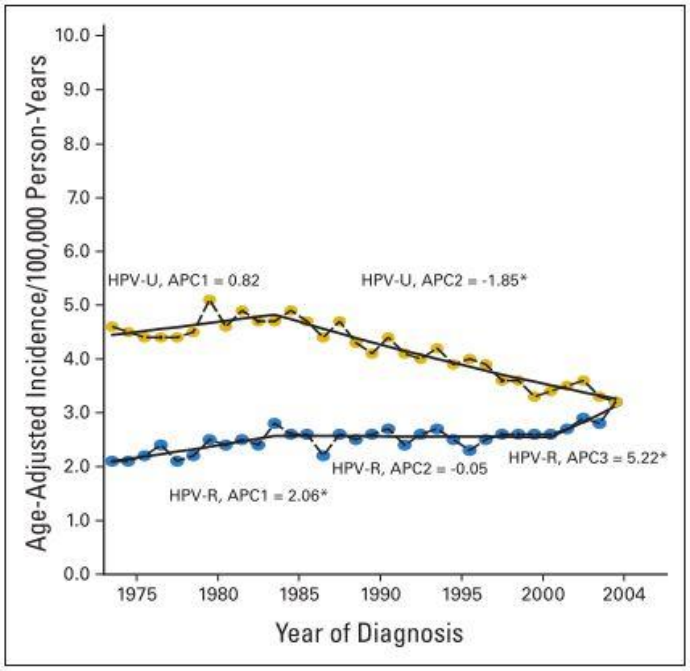

Figure 1: This well-known figure shows the increasing incidence of tumors arising from HPV-related locations (HPV-R) in comparison to that belonging to HPV-unrelated locations (HPV-U) (3)**.**

HUMAN PAPILLOMAVIRUS INFECTION: A REVIEW

Human papillomavirus was first discovered in basal keratinocytes in the skin or mucosal membranes in the 1950s, but it was later on that its carcinogenic power was described by Harald zur Hausen, for which he received the 2008 Nobel Prize in Medicine.

The carcinogenic implications of HPV are diverse and well known: cervix, anus, penis, vulva, vagina, and oropharynx.

When analyzing cervix infection, HPV is recognized to be transmitted mainly via sexual contact (through minor injuries of the skin or mucous membrane). HPV exposure is extremely common (up to 43-62% in the United States in 2011) (6), with similar data in Spain from a report by the Health Ministry in 2013 (7). Despite this, it is mainly an asymptomatic infection. The most part of people exposed to it will clear the virus and never develop carcinoma.

On the other hand, we can gather less knowledge about the mechanism of transmission of the HPV on the upper airways, but there seems to be a relation between oral sex and HPV infection in the oral cavity (8).

Pickard et al (9) showed that at any given time, 7% of the US population between 18-30 years has a prevalent oral/oropharyngeal HPV infection, but only half of them will create antibodies.

An interesting study led by Gillison concluded that men are more likely than women to have an oral HPV infection (10). It has been hypothesized that this may be due to the female genital mucosa having a higher HPV viral load than the male genital mucosa/skin (11).

As some of the studies cited suggest, the risk of oral HPV infection increases with the number of oral sexual partners (12). This fact, added to the decrease in the age of sexual debut and increase in the number of sexual partners, may have contributed to a rise of the rate of oropharyngeal HPV exposures in developed countries.

Oropharyngeal infection by HPV is a necessary event in the development of HPV-related oropharyngeal squamous cell carcinoma (OPSCC), but given the high prevalence of infection, we assume that most of these contagions do not progress to cancer. Besides, there is evidence that HPV infection likely precedes the development of OPSCC by many years, if not decades (13).

Despite this, and the fact that the natural history of oral HPV infection is not well defined, the recent increased incidence of HPV-positive squamous cell carcinoma of oropharynx leads us to believe that this two facts (infection and development of cancer) may be related, and reflect societal changes in sexual behavior that have occurred over time in the developed world (14).

VACCINATION

Recently, a prophylactic HPV vaccine has been included in national immunization programs of most developed countries, with the goal of preventing cervical and other non-cervical (such as oropharyngeal) HPV related cancers (15).

In Spain, a recent review of the Vaccination Program against VPH by the Health Ministry (7) has gathered very positive data in relation to the duration of the protection offered by the vaccines, and has stated that the inclusion of the vaccination against VPH in preadolescent girls meets criteria for a cost-effective intervention in our country.

There are two vaccines on the market, Gardasil © (quadrivalent) and Cervarix © (bivalent), both protecting against HPV types 16 and 18 (a detailed exposition of the biology of the HPV can be found later in this text). The Spanish Pediatrics Association recommends universal vaccination for all preadolescent girls (16).

In some countries, such as United States, there has been an entrance of males into vaccination programs, due to the cases of HPV-related cancer, primarily head and neck, as we have seen, but also because of the rates of anal cancer between homosexual males (1).

In the future, the currently available vaccines may also show promising results on preventing HPV-positive OPSCC caused by HPV 16.

Unfortunately, the prophylactic vaccine is not effective on established infections and cancer lesions, so the study of a therapeutic HPV vaccine to treat HPV-related cancer remains an area of crucial importance.

BIOLOGY OF HPV AND ONCOGENESIS IN THE OROPHARYNX

Ever since mammals evolved skin glands and hairs, their epithelia and mucosa have been infected by a plethora of papillomaviruses, whose infectious cycle is linked to the differentiation program of the keratinocyte. HPV belongs to a family of small, non-encapsulated viruses that infect warm-blooded vertebrates. In this section, we will examine the basics on HPV biology and oncogenic mechanisms.

HPV is an epitheliotropic, non-enveloped DNA virus that carries a single molecule of circular double-stranded DNA. The genome is broken down into three regions which consist of a long control region (LCR), an early (E) region and a late (L) region.

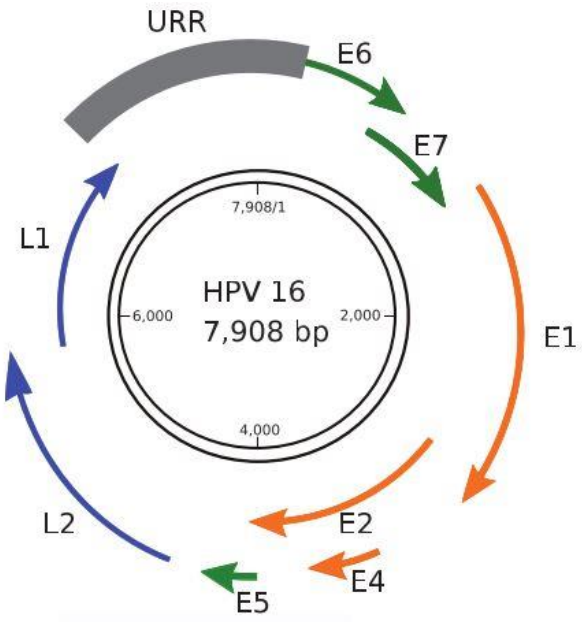

Figure 2: This scheme summarizes the functions of the HPV genes, exemplified on HPV16. In green, we can see genes implicated in oncogenesis; orange shows viral replication genes; blue is used for viral capsid genes (17)**.**

At present, more than 200 genotypes of papillomaviridiae have been identified. They can be classified regarding quite different aspects, but our focus will be oncogenic-power oriented, following the International Agency for the Research on Cancer Classification:

- High risk types: **16**, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82.
- Low risk types: **6**, **11**, 40, 42, 43, 44, 54, 61, 72 and 81.

In the head and neck area, there is a predilection for HPV-positive tumors to occur in the reticular crypt epithelium of palatine and lingual tonsils and head and neck sites with mucosa associated lymphoid tissue. It is possible that this occurs due to the particular microanatomy of the crypts, while some other theories are proposed (18). The precise nature of the cell surface receptor/s that allow for the initial attachment to the cell remains disputed.

90% of HPV infections are cleared within two years (although high risk HPV tends to persist longer) (19), but once immune evasion is established, a critical step for malignant transformation occurs: the integration of HPV DNA into the cellular genome of the tonsil crypt epithelium.

Two HPV genes, E6 and E7, produce two oncoproteins (with identical name) that confer the virus with oncogenic potential through their inhibitory effects on p53 and retinoblastoma (Rb) proteins:

E6	This oncoprotein is a proteolysis inductor, leading to degradation of the tumor suppressor p53. As p53 usually facilitates repair to damaged host DNA by arresting cells in the G1 phase (or else inducing apoptosis), E6 expressing cells face increased mitotic stress and genomic instability (20).
E7	It causes cell cycle disturbance by binding and inactivating tumor suppressor proteins of the retinoblastoma (Rb) family, thus causing cell proliferation through abnormal entry into the S-phase (18). Rb protein normally prevents cells from entering S phase of the cycle, so its inactivation promotes cellular cycle progression, allowing the differentiating keratinocyte to enter uncontrolled proliferation.

The inactivation of the retinoblastoma family by the E7 oncoprotein is a key event, since it also results in overexpression of p16 tumor suppressor protein, allowing the use of p16 as a surrogate marker for HPV-related oncogenesis (21).

P16 is a cyclin-dependent kinase inhibitor that prevents progression from G1 phase to S phase by inhibiting cyclin-dependent kinases “4” and “6”. These kinases phosphorylate Rb and decelerate the cell cycle progression (see *Figure 3*). Studies have shown that in normal conditions Rb regulates p16 presumably through a negative feedback mechanism (22), so when Rb is inactivated due to the expression of E7, p16 is overexpressed, in order to restore the physiological balance that keeps the cell from entering the S phase, thus giving us the possibility to use it as a surrogate marker of E7 expression.

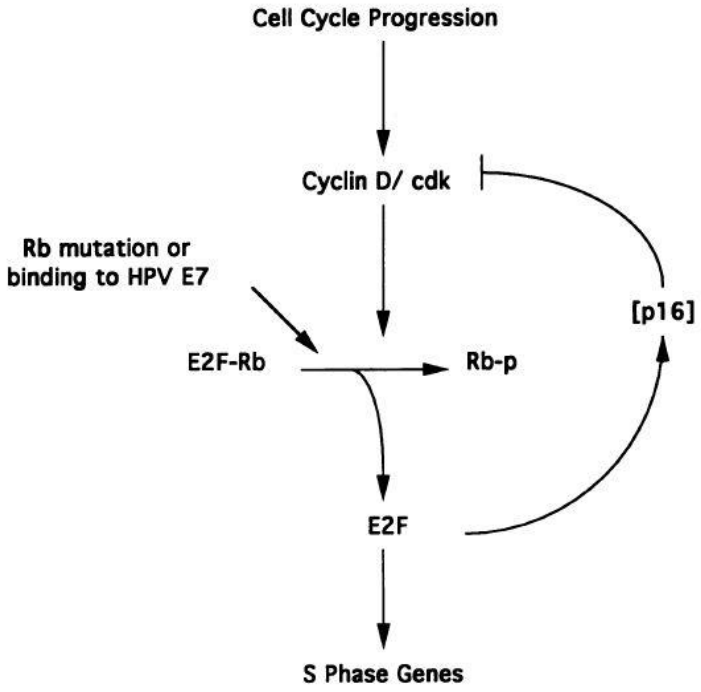

Figure 3: A proposed model explaining the regulation of a p16-related CKI activity by the level of active Rb. When there is functional loss of Rb as a consequence of viral E7, p16 increases its activity (22)**.**

The role of other factors in the development of HPV-positive OPSCC is unclear:

- Smoking is a risk factor for reduced clearance of oral HPV infection, as HIV is, and therefore tobacco exposure and immune status may play a role in the development of HPV-positive OPSCC.
- Other risk factors for the development of OPSCC, such as genetic polymorphisms, may also have an underestimated value.

DIAGNOSIS OF HUMAN PAPILLOMAVIRUS INFECTION

Unlike cervical cancers, in which HPV is a necessary event, viral DNA detection in oropharynx cancer may have two possible meanings:

- It may be a transitory infection in replicative phase, in which the virus does not carry a relevant role in the carcinogenesis.
- It may be an infection with a defined oncogenic role.

Despite the escalating expectation for a reliable determination of HPV status, there is not yet a standard strategy for HPV detection in head and neck cancers (they vary not just in design, but in their detection targets). We shall remember that the ultimate value of any HPV detection strategy lays in its ability to both recognize the presence of HPV and discern its potential as a driving force of tumorigenesis (23).

Over 90% of HPV-related OPSCC are caused by HPV type 16, so any strategy for diagnosing HPV on Head and Neck cancer must include at least this type.

Next, a brief summary of the main disposable diagnosis techniques will be exposed. On page 11, a table synthetizes the key points of these methods.

Microscopic evaluation:

HPV-positive OPSCCs have a distinctive appearance, thus facilitating the interpretation of HPV testing in those instances where there is a disparity between the morphologic findings and a test result.

PCR:

- HPV DNA detection with PCR: This technique owns an incomparable sensitivity, but the value of detecting HPV at very low levels is offset by other factors that confound the biological and clinical relevance of viral detection.
- HPV RNA detection with PCR: The detection of E6/E7 messenger RNA is the current gold standard for clinically relevant HPV. The presence of E6/E7 mRNA indicates that HPV is not merely present, but is transcriptionally active. Unfortunately, it remains a challenging technique whose use is mainly restricted to the research laboratory.

DNA in situ hybridization (ISH):

It is a signal amplification technique that utilizes labeled DNA probes complementary to targeted viral DNA sequences. It allows visualization of DNA in the histologic context.

Direct comparison of DNA ISH and PCR-based methods suggests that DNA ISH may be a preferable HPV detection tool for both practical and biological considerations (23), but this is yet to confirm. DNA ISH has somewhat limited sensitivity, especially for tumor samples with low viral copy numbers.

P16 inmunohistochemical staining as a surrogate marker of HPV:

This technique shows excellent performance in HPV detection (similar to that achieved by ISH), but it has better features regarding simplicity and cost.

As we have explained, the rationale for p16 testing as a surrogate for transcriptionally active HPV is a consequence of viral oncoprotein E7 binding to the retinoblastoma protein and its subsequent degradation. In the absence of the retinoblastoma protein, tumor cells are free to replicate in the presence of p16, which now accumulates, leading to high expression that can be detected by routine immunohistochemistry.

Due to the characteristics of our Hospital, this technique will be the one performed in our study. In point 7.5 of this protocol (Variables), a detailed description of the process as performed in Hospital Universitari Dr. Josep Trueta can be found.

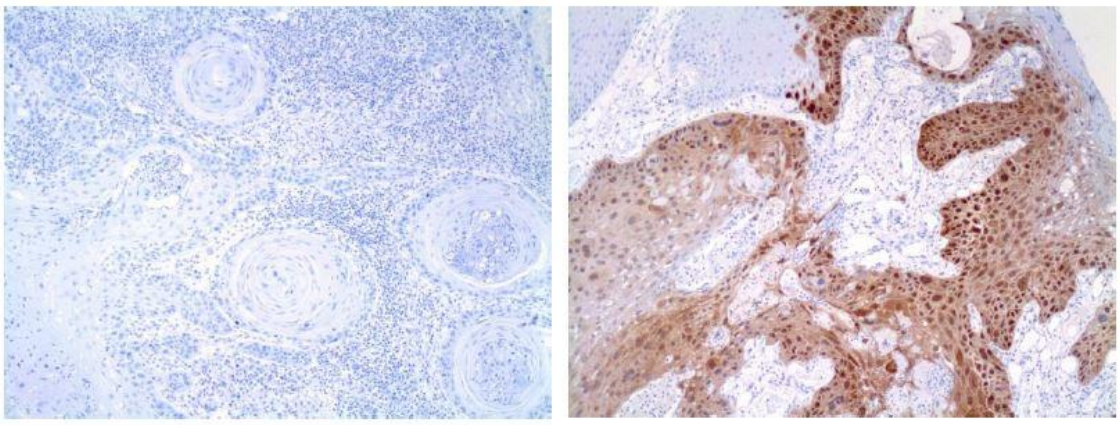

Figure 4: Example of p16 inmunohistochemical positivity. Negative p16 staining on the left, and positive p16 staining on the right (24)**.**

The future: multi-modality HPV analysis:

Use of p16 staining as standalone test for HPV detection is associated with a small false positive rate. Multimodality detection strategies look forward to utilize a more rigorous HPV-specific detection assay such as HPV-DNA in situ hybridization and/or PCR-based assay, for those patients that are classified as p16 positive.

Comparison of the different HPV testing techniques		
Method	**Advantages**	**Disadvantages**
Microscopic Evaluation	Low cost. Serves as a backing up for other HPV tests.	Not enough specific to be used alone.
PCR for HPV DNA	High sensitivity.	Unable to distinguish biologically relevant from irrelevant infections.
PCR for E6/E7 HPV mRNA	High sensitivity. High specificity (Gold Standard for regarding a tumor as HPV-related).	Technically challenging. Requires fresh frozen tissue.
DNA in situ hybridization	High specificity. Easy to integrate into Pathology laboratory.	Low sensitivity at low copy numbers. May be difficult to interpret.
P16 IHQ	High sensitivity. Low cost. Widely available in most laboratories.	Not highly specific. Must be correctly interpreted.
P16 + another method	Allows combining the high sensitivity of p16 with a more HPV-specific method. Gives insight into the biologic significance of detected HPV.	Increased cost

Table 1: Comparison of HPV testing techniques (25)**.**

CLINICOPATHOLOGIC FEATURES OF HPV-ASSOCIATED HEAD AND NECK CANCER

Epidemiological factors

As exposed earlier, HPV-positive tumors tend to belong to younger patients, with a median of diagnosis of 54 years, less exposure to tobacco and alcohol, and higher socioeconomic status and education (3). HPV positivity is less frequent in Afro-Americans than in Caucasians, with a threefold higher incidence in males than females (1).

This male predominance exhibited cannot be fully explained by difference in sexual behaviors, which suggests potential biologic differences between men and women that need further investigation.

Sexual behavior

HPV infection appears to be a sexually-acquired disease, mainly through oral sex behavior, but some studies have even proposed that open-mouthed kissing may also have a role in the development of oral colonization (12). Vaginal sex was not found to be related to the development of oral HPV infection. Some studies suggest that certain sexual behaviors can be seen as surrogate for HPV-16 exposure (18).

Prospective studies are needed to further evaluate the risk of incident oral HPV infection associated with each specific type of sexual behavior.

It is possible that in addition to HPV infection, there are other cofactors such as genetic susceptibility to integrate the virus, or nutritional factors, or tobacco/alcohol interaction that have an important role in cancer onset.

The typical patient is framed as follows: Multiple sexual partners and/or orogenital sexual partners, plus early sexual debut.

Anatomical sites

The preference of HPV for the oropharynx is unexplained (it may be related to the transitional mucosa found in the tonsillar tissue, which shows similarities to the cervical mucosa). Please, check *Annex 1* for a quick review of the anatomical landmarks of the oropharynx.

Biological profiles

HPV-positive tumors may harbor fewer or different genetic alterations, which can be associated with better response to therapy. They also have higher radiosensitivity, probably due to intact apoptotic response to radiation, and immunologic response, because of stimulation of immune response directed to viral specific tumor antigens (26).

Clinical stage at presentation

HPV-positive tumors are more likely to present with early T stage, and higher N stage (usually cystic and multilevel), and have distinct histological features. Because small primary oropharyngeal tumors are unlikely to be symptomatic, most patients seek medical attention due to symptomatic nodal disease (27).

The incidence of distant metastases seems to be lower in HPV-tumors (1), but in case they appear, the location is in nontraditional sites and they present later than usual (more than 2 years after initial treatment). These differences require physicians to be vigilant about unusual complaints in patients with a history of HPV-positive OPSCC.

Prognosis

HPV-positive oropharyngeal cancer has an improved overall and disease-free survival, compared to patients with HPV-negative oropharyngeal cancer patients (28). HPV-positivity confers a 60-80% reduction in risk of death from cancer compared to similarly treated HPV-negative tumors. The absolute survival difference between HPV-positive and negative tumors is consistently higher than 30% across prospective studies (29).

This facts hold true with different diagnosis techniques (PCR, in situ hybridization, or immunohistochemistry), and after adjustment for differences in favorable prognostic factors associated with HPV positive patients (younger age, better performance status, fewer comorbidities, less smoking…). The exact mechanism behind this improved survival is unclear, but it has transitioned OPSCC from one of the gravest diagnoses of carcinomas of the upper aerodigestive tract to the best (because of a dramatic improvement in the 5-year survival rate for HPV-negative OPSCC).

CHARACTERISTIC	HPV-Positive	HPV-negative
Anatomical site	Tonsil, base of tongue, uvula, soft palate, posterior and lateral pharyngeal wall	All sites
Demographics	Younger Higher socioeconomic status	Older, lower socioeconomic status
Risk factors	Sexual behavior	Alcohol and tobacco use
Incidence	Increasing	Decreasing
Histology	Nonkeratinized, basaloid, poorly differentiated	Keratinized
Stage at presentation	Early T, more advanced N Lymph nodes are often cystic	Variable
Molecular/pathological changes	TP53 wild-type P16 positive No EGFR overexpression	TP53 mutated High EGFR expression
Survival	Improved	Unchanged
Second primary tumors	Less common	More common

Table 2: Differences between patients with HPV-positive and HPV-negative SCCHN (30)**.**

IMPACT OF HPV ON CLINICAL MANAGEMENT

The standard treatment for OPSCCs at present is mainly dependent on the stage of the disease and patient/clinician preferences (30):

- Early disease (T1-2, N0): Single-modality treatment (in the form of surgery or radiotherapy) is usually recommended.
- Advanced stage disease: Chemoradiotherapy with or without neck dissection, or surgical resection with reconstruction and postoperative chemoradiotherapy, as required.

One obvious question arises: We have stated that HPV-related OPSCC is accompanied by a more favorable outcome, so should the current classification system for HNSCCs be altered to reflect different status of HPV infection? Should a treatment de-escalation protocol be performed in order to avoid these HPV-positive patients their share of side effects?

These are some of the emerging options for HPV-related OPSCC: reduction in RT dose, alterations in target volume, omitting concurrent chemotherapy, replacing chemotherapy with biologicals…

The data presented suggest that HPV status could be used in the clinical decision-making processes to select patients for less aggressive treatment, but is it a reality in nowadays clinical practice?

TREATMENT DE-ESCALATION: HOW PERTINENT IS IT?

There are a number of ongoing clinical trials dealing with the question stated above: De-ESCALaTE (NCT01874171), ADEPT (NCT01687413) and E3311 (NCT01898494) are just a few examples. Most of the de-escalation protocols favor reduction in radiotherapy intensity, since the radiation component of concurrent therapy is the most toxicity-producing.

Current recommendations are to treat patients according to their stage of disease at presentation, irrespective of HPV status. Despite this, according to what we have tried to elucidate, patients with OPSCC should be encouraged to enroll on clinical trials specifically targeting HPV-positive and HPV-negative cohorts. This door leads to a future in which HPV-positive oropharyngeal cancers may be treated with less intense treatment strategies that do not compromise survival outcomes, but lower the risk of side effects.

Potential long-term side effects of current chemoradiation include dysphagia, xerostomia, feeding-tube dependency from fibrosis and scarring of the pharyngeal muscles, chronic aspiration and chronic fatigue. There is, therefore, an ethical need of adapting current treatments to the real requirements of HPV-positive patients with oropharyngeal squamous cell carcinoma.

3.2 JUSTIFICATION

Up to date, clinical practice does not incorporate the systematic determination of p16 status in patients that are diagnosed of oropharyngeal squamous cell carcinoma (OPSCC). In our Hospital, there is a lack of knowledge of the current rate of this group of patients that are infected by HPV.

As most epidemiological information about this issue comes from studies from abroad, there is an urgent need for investigating if the data argued above can be extrapolated to our population. Sexual behavior may vary from the United States to Girona, so there are reasons to suspect that the rate of HPV infection in oropharyngeal cancer in our population may be different.

The relevance of HPV testing is huge: it is by no means restricted to better prognosis, but, as we have seen, the direction of current clinical trials points into a selection of patients for specific therapies based on HPV-tumor status. We can, therefore, insight that p16 determination will become part in the near future of the regular diagnosis tests that are performed on every patient that suffers from OPSCC.

A huge change in the approach of the treatment for these patients is about to happen, and we need to be prepared for it, gathering as much information about this population as possible.

The data collected by our cross-sectional study will supply the multi-disciplinary team that works in the Head and Neck Committee in Girona with a global picture of the epidemiological information that concerns this type of patients. This is not a triviality, since we are talking about a real epidemic: Chatuverdi et al have estimated that by 2020 the incidence of HPV-positive OPSCC will be greater than the incidence of cancer in the cervix, and by 2030 half of all head and neck cancers will be related to HPV (31).

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5. HYPOTHESIS

The presence of human papillomavirus infection in patients diagnosed of oropharynx cancer is a relevant predictor of prognosis, whose prevalence is not studied in our population.

6. OBJECTIVES

MAIN OBJECTIVE

To determine the prevalence of human papillomavirus (HPV) infection among patients diagnosed of oropharynx cancer in the province of Girona.

SECONDARY OBJECTIVES

- To analyze possible associations between the infection by human papillomavirus and other epidemiological aspects that are observed in our population of study.
- To analyze the relation between the presence of the virus and the stage at which the tumor is diagnosed.

7. MATERIALS AND METHODS

7.1 STUDY DESIGN

This protocol is designed as a cross-sectional study, since our objective is to describe the prevalence of human papillomavirus (HPV) infection among patients diagnosed of oropharynx cancer in our hospital. It will be performed in the Otorhinolaryngology Department at Hospital Universitari Dr. Josep Trueta in Girona during a period of time of two years.

7.2 STUDY POPULATION

The study population will include adult patients that are diagnosed with oropharyngeal cancer in Hospital Universitari Dr. Josep Trueta. Patients that are suspected to suffer from a tumor in the head and neck area are conducted through a "quick diagnosis" circuit that ends up in a committee known as *Head and Neck Functional Unit*, a multidisciplinary team that decides which is the most suitable treatment for each one of these patients. Thereby, every patient diagnosed with an oropharyngeal tumor will end up in this committee, having participated in our study first.

It is important to underline that our target population includes only new oncologic diagnoses since the beginning of our recruitment; there will be no use of previous data collected in our Hospital.

7.3 INCLUSION AND EXCLUSION CRITERIA

INCLUSION CRITERIA

- Patients willing to cooperate in the study, thus giving written informed consent (see *Annex 4* for more information about the informed consent).
- Patients aged 18 years or older.
- Patients diagnosed with cancer of the oropharynx (tonsillar, base of tongue, soft palate, uvula, posterior pharyngeal wall and lateral pharyngeal wall).

EXCLUSION CRITERIA

- Patients whose diagnosis of oropharyngeal squamous cell carcinoma was prior to the beginning of the recruitment for the study.
- Patients with a history of cancer of any other cellular lineage in the oropharyngeal area.
- Contraindications to perform biopsy of the tissue.

7.4 SAMPLE

SAMPLE SELECTION

A non-probabilistic consecutive sampling will be performed, since we will include in our study every patient diagnosed with oropharynx cancer in our unit that is willing to cooperate.

SAMPLE SIZE

In order to calculate our sample size, we used the GRANMO Calculator. According to the data of the Head and Neck Functional Unit, an average of 35 patients with oropharynx cancer are diagnosed every year in Hospital Universitari Dr. Josep Trueta. Our revision of the available bibliography has made us decide a 0.03 estimation of the proportion in the population (that is, 30% of all oropharyngeal cancers are expected to be caused by HPV). Accepting an α risk of 0.95, and with a foresight of 0 dropouts, our sample will be composed of 51 patients.

ESTIMATED TIME OF RECRUITMENT

The estimated time of recruitment for assessing the figure proposed (51 cases) is approximately two years. Anyway, we will possibly exceed this number, since the annual record of cases diagnosed in our Hospital is higher.

7.5 VARIABLES

OUTCOME VARIABLE

Our variable of interest, or outcome variable, is the **presence of human papillomavirus infection in our population**, determined by the p16 result (positive or negative), which is used as a surrogate marker of infection. It is a nominal dichotomous qualitative variable.

Of the four techniques previously mentioned, microscopic evaluation and p16 determination through immunohistochemistry are the only ones available in our Hospital (see point 9: Limitations). As explained later in the work plan, a biopsy of the lesion will be obtained through fiberoptic endoscopic evaluation in the context of the study of the patient, and a p16 determination will be performed by the Pathology Department.

The next few lines will be dedicated to describe this inmunohistochemical procedure, by which we define the outcome variable of our study.

Determination of p16 through immunohistochemistry

Immunohistochemistry refers to the technique that allows the detection of antigens (p16 expression in our particular case) in cells of a tissue by taking advantage of the principle of antibodies binding specifically to antigens in biological tissues. It is a simple and elegant idea that emerged during the '50s and expanded the capacity of diagnosis of a Pathology laboratory.

The objective of immunohistochemistry is to detect, amplify and to confer visibility to a particular antigen. For the detection of this antigen, a "primary antibody" is used. Then, in order to amplify this reaction, a "secondary antibody" joins in scene, reacting against the "primary antibody". Finally, the visualization of the whole complex is made possible through a conjugated enzyme (peroxidase) that catalyzes a colour-producing reaction.

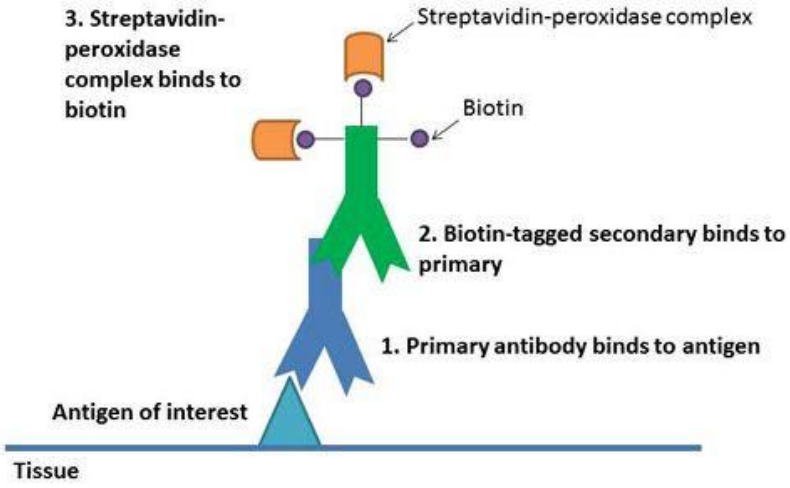

Figure 5: The primary antibody reacts against the antigen of interest. Then, the secondary antibody binds to the primary one (32)**.**

Every secondary antibody is tagged with a number of biotin molecules, that have high affinity for the streptavidin, so multiple streptavidins get bound to each secondary antibody, which amplifies the signal. These streptavidins also have peroxidase enzymes conjugated to them, and it's these enzymes that will help cause the colour change in our DAB chromagen.

These are the main steps of the immunohistochemistry process, as performed in the Pathology Department of the Hospital Universitari Dr. Josep Trueta:

- Fixation of the tissue in a 10% formaldehyde solution, in order to preserve the material.
- Sectioning: 3µ sections are prepared.
- Heating of the samples, during 1h at 60ºC.
- Insertion of the samples in the VENTANA System (automated proceedings):
    - Removal of paraffin leftovers.
    - Buffer washing.
    - Antigenic recovery by breaking formalin bridges (by applying heat and CC1 solution).
    - Peroxidase inhibition, in order to diminish false positives in relation to false reactivity due to this endogen enzyme.
    - Incubation of the primary antibody (anti-p16 antibodies).
    - Incubation of the secondary antibody (the one that will amplify the reaction; it is directed against the primary antibody) and the enzymes (peroxidase in this case).
    - Visualization through a revealing substance named DAB (chromogenic): When the DAB interacts with oxygen, it acquires its characteristic brown color (see *Figures 4* and *6* in this text).
    - Counterstaining with hematoxylin, so that the Pathologist can lean on anatomical references when giving out the diagnosis.

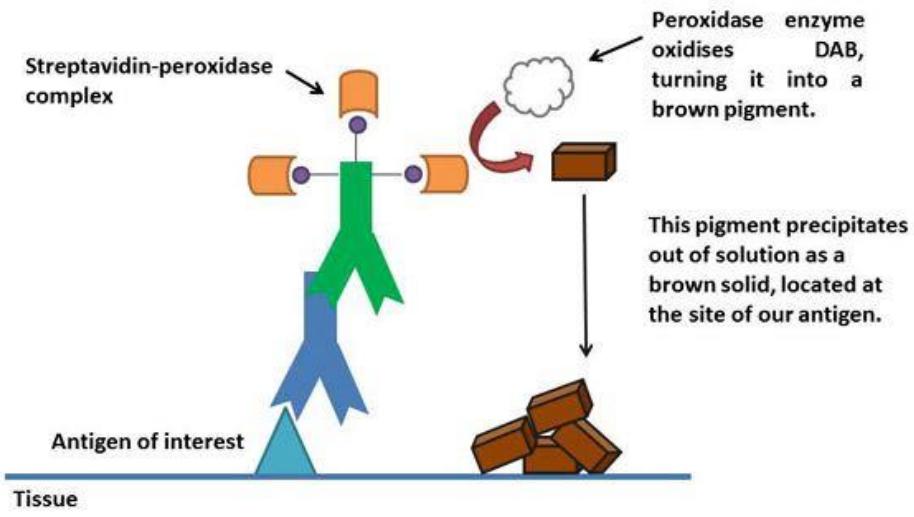

Figure 6: The peroxidase enzymes react with the DAB: it gets oxidated and forms a solid pigment which precipitates where our antibodies are (32)**.**

INDEPENDENT VARIABLES

Our study is designed as a cross-sectional one, bringing forth the impossibility to define one single independent variable, as every epidemiological aspect we may want to study will be considered as one. Therefore, we will define these aspects in the way of covariates.

COVARIATES

We are managing two groups of covariates: those related to epidemiological information, that will be collected through a data collection form (see *Annex 2*), and those related to the tumor itself (these covariates are the result of clinical procedures, such as laboratory data, image techniques…).

The next list offers the covariates that will be collected in order to analyze its relations regarding the outcome variable. A brief summary presented as a table stipulates the method of measurement of each one:

- **Age** (discrete quantitative variable): As expressed in years at the moment of diagnosis, measured in one year-intervals since the date of birth. The age of the patients will be consulted from the ID card or any other official document facilitated to the investigators.
- **Gender** (nominal dichotomous qualitative variable): Male/female. Data will be collected from the ID card of the patient or any other official document facilitated to the investigators.
- **Ethnicity** (nominal qualitative variable): four groups will be considered (Caucasian, African, Asian and Latin-American), depending on the investigator's judgment.
- **Smoking**: It will be analyzed as a nominal qualitative variable, in terms of non-smoker, smoker and ex-smoker.
- **Alcohol consumption** (qualitative variable): defined as no intake, moderate intake, and heavy intake.
- **Prior history of oral sex** (qualitative variable).
- **Primary site of the tumor at enrollment** (qualitative variable): considering tonsillar, base of tongue, uvula, soft palate, posterior pharyngeal wall and lateral pharyngeal wall. The location will be assigned by the investigators given the physical examination and inspection of the lesion through video-endoscopy, also taking into account the image techniques.
- **T stage at enrollment** (ordinal qualitative variable).
- **N stage at enrollment** (ordinal qualitative variable).
- **M stage at enrollment** (ordinal qualitative variable).
- **Histological tumor grade** (ordinal qualitative variable). The Pathologist will classify the grade of the tumor considering the differentiation of its cells, following the Broder classification for carcinomas.

	Variable	Description	Method of measurement
Outcome variable	P16 positivity	Nominal dichotomous qualitative	Immunohistochemistry technique performed on the biopsy tissue
Covariates	Age	Discrete quantitative	Data collection sheet (information from the ID of the patient)
	Gender	Nominal dichotomous qualitative	Data collection sheet (information from the ID of the patient)
	Ethnicity	Nominal qualitative	Data collection sheet
	Tobacco	Nominal qualitative	Data collection sheet
	Alcohol	Nominal qualitative	Data collection sheet
	Oral sex history	Nominal qualitative	Data collection sheet
	Location of the primary tumor	Nominal qualitative	Direct visualization and image techniques (MRI, CT)
	T stage	Ordinal qualitative	Image techniques, pathology exam
	N stage	Ordinal qualitative	Image techniques, pathology exam
	M stage	Ordinal qualitative	Image techniques, pathology exam
	Histological tumor grade	Ordinal qualitative	Microscopic evaluation by the Pathologist

Table 3: Summary of the different variables taken into account in our study

7.6 DATA COLLECTION AND STUDY CIRCUIT

The first visit of a patient to the Head and Neck Functional Unit serves as a starting point to the data collection of our study.

On the first visit, a patient with a suspicion of cancer of the otorhinolaryngological area is examined by a doctor of our team:

- A detailed anamnesis is performed, concerning the time of progression of the lesion/symptoms and some data related to the covariates that will be collected hereafter.
- A physical examination, with special emphasis on the palpation of possible lymphadenopathies, is also suitable.
- Direct examination of the cancerous lesion through laryngoscopy will indicate the doctor the need of a biopsy.

A video-endoscopy on a subsequent appointment will allow the physician to perform a biopsy of the suspicious area under local anesthesia.

The tissue sample that results from the biopsy is conducted to the Pathology Department of the Hospital, where a p16 determination through immunohistochemistry will be performed by our team, in addition to the usual microscopic evaluation that will label the lesion as a squamous cell carcinoma in most of the cases.

It is important to underline the fact that, during the time of recruitment of the study, every biopsy concerning the oropharyngeal area that is performed in our department will include a p16 determination, therefore expediting the procedure of entering the study. This is specified on the *Phase 1: Coordination* in the Work Plan, that is described later on in this text.

It is in this point of the process that out team will be certain that the lesion meets criteria for the inclusion in our investigation, proceeding then to ask for a written consent to the patient. An information document (see *Annex 3*) will be handed to him or her, and the investigator will make sure that the patient understands the terms and agreements of the investigation.

When the informed consent is signed, the investigator and the patient will fill in the Data Collection Sheet presented in *Annex 2*, in order to recover all the covariates of interest. This document will only include an identification number for each patient, with no personal data in sight, in order to maintain the anonymity and confidentiality of the process.

A neck CT scan is requested on every patient in order to establish the TNM stadium of the cancerous lesion (in cases of ill-defined margins a MRI will be needed). A chest-CT scan will assess the presence or absence of metastases. This data will be included in the Data Collection Form as well, and its significance lies, as we have discussed, in the possible differences observed among HPV-positive and negative tumors.

In the course of 15-21 days since the first visit, every case is displayed in a meeting of the *Head and Neck Committee*, which decides the best treatment for each case, from a multi-disciplinary standpoint.

When the data from the 51 patients included in our sample is collected (in an estimated time of two years), the data analysis phase begins. The whole process will end up on May 2018, when the publication and dissemination of the results begins.

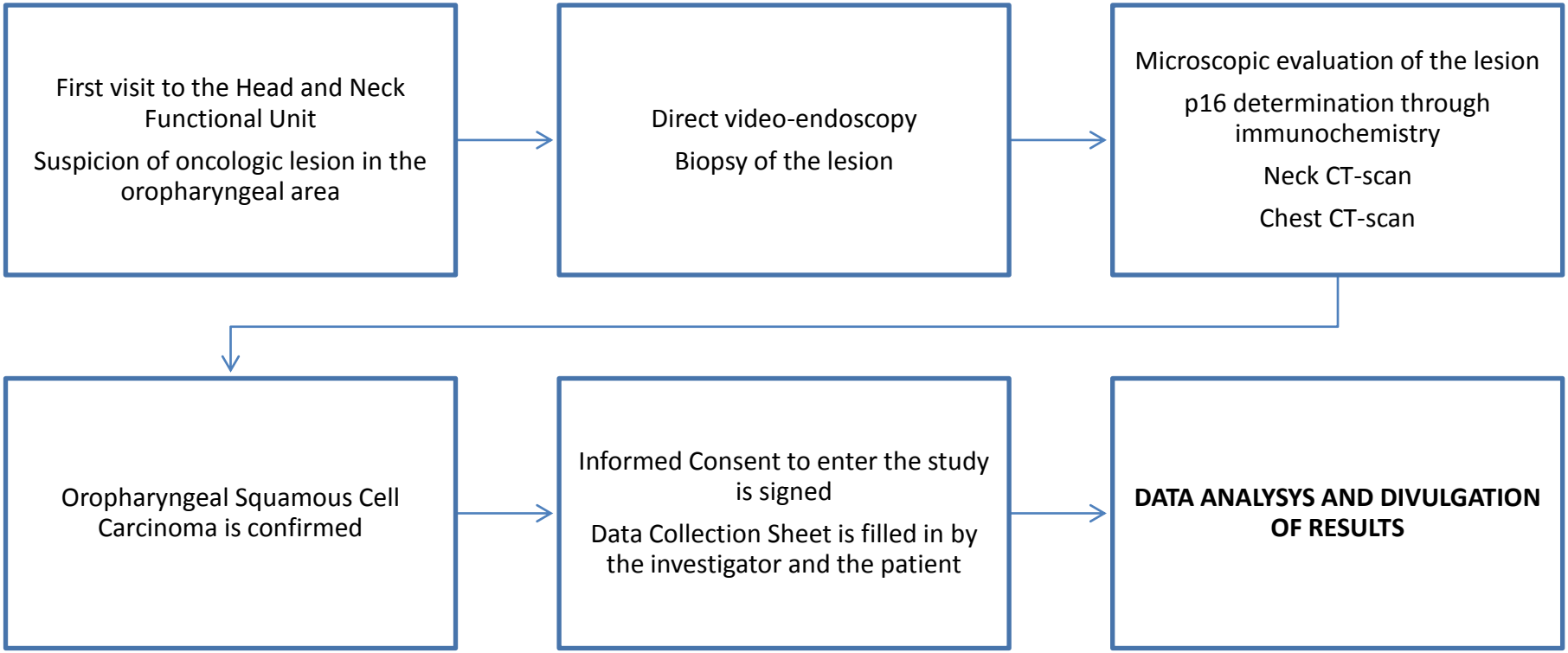

Figure 7: Flowchart containing the highlights of the study

7.7 STATISTICAL ANALYSIS

Our study will include three levels of statistical analysis of the data collected: univariate analysis, bivariate analysis and multivariate analysis.

Univariate analysis:

Due to the characteristics of a cross-sectional study, we cannot define our variables in terms of dependent and independent. However, we consider the p16 positivity as the outcome variable (qualitative variable), and we will study its prevalence in relation to a group of covariates.

For qualitative or categorical variables, results will be expressed as percentages. For quantitative variables, assuming that they are not normally distributed, a median will be estimated. In case that they follow a normal distribution, an arithmetic mean and a standard deviation will be calculated.

Bivariate analysis:

Percentages for categorical variables will be shown in a contingency table, and a Chi-square test (χ^2) will be used to compare the different epidemiological data between both groups of patients.

For the analysis of the relationships between our variable of interest (VPH infection, which is qualitative) and quantitative variables, a Mann-Whitney test will be used for the non-normally distributed ones. For normally distributed variables, a Student's T test will be performed.

Multivariate analysis:

The analysis will be adjusted for covariates that are statistically significant ($p<0.05$). A logistic regression analysis will be performed to assess the association between both groups of patients (those with HPV infection, and those without it) and the different covariates.

8. WORK PLAN AND CHRONOGRAM

8.1 SCHEDULE

The main landmarks of our study are described below, and a chronogram is presented on the next page for easy visualization of the whole process. The following sequence of activities will be carried out by the main investigator and his team:

- PHASE 1: Preparation and Coordination (November 2015 – February 2016).
    - **Activity 1**: Protocol elaboration and evaluation by the Ethical Committee of our Hospital. The objectives and covariates chosen as a starting point for our study are the result of a clinical need detected by the clinical researchers and a bibliography revision of the subject.
    - **Activity 2**: Coordination of the research team. The need of systematic determination of p16 on every patient with a suspicion of oropharyngeal cancer during phase 2 (from March 2016 to March 2018) has to be agreed by the whole ORL team and the Pathology laboratory.
- PHASE 2: Field work and data collection (2 years: March 2016 – March 2018).
    - From March 2016 to March 2018, the recruitment of patients will be performed. We expect to meet the required sample size of 51 patients during this phase.
    - The data collected on the Form presented in *Annex 2* will be digitalized and saved waiting for future analysis.
- PHASE 3: Data analysis and final evaluation (April 2018 – May 2018).
    - A statistician will be involved in the study so that statistical analysis is performed with excellence guarantees.
    - A meeting of the investigation team will serve to interpret the preliminary results.
- PHASE 4: Publication and dissemination of the results (June 2018 – October 2018).
    - The results will be presented in a number of congresses, including:
        - The Catalan Otorhinolaryngology Associations (SCORL).
        - European Academy of Otorhinolaryngology, Head and Neck Surgery (EAROL-HNS).

8.2 CHRONOGRAM

TASKS	2015		2016												2017												2018										PERSONNEL
	N	D	J	F	M	A	M	J	JL	A	S	O	N	D	J	F	M	A	M	J	JL	A	S	O	N	D	J	F	M	A	M	J	JL	A	S	O	
PHASE 1: PREPARATION AND COORDINATION																																					
Protocol elaboration and evaluation																																					Main researcher
Coordination of the research team																																					ORL team at H. Josep Trueta
PHASE 2: FIELD WORK AND DATA COLLECTION																																					
Patients recruitment																																					ORL team
P16 determination																																					Pathology team
Epidemiological data collection																																					Clinical researchers
PHASE 3: DATA ANALYSIS AND FINAL EVALUATION																																					
Statistical analysis																																					Statistician
Interpretation of the results																																					Main researcher
Final report and evaluation																																					
PHASE 4: PUBLICATION AND DISSEMINATION																																					
Scientific publications																																					Main researcher
Attendance to SCORL and EAORL-HNS congresses																																					ORL team

9. STUDY LIMITATIONS

The following lines will serve as a brief exposition of the possible limitations that may interfere in our research protocol.

As discussed in the introduction, p19 overexpression is the result of the inactivation of the retinoblastoma family by the E7 oncoprotein. We use p16 as a surrogate marker for HPV-related oncogenesis. The simplicity, low cost and high sensitivity of p16 immunohistochemistry (sensitivity, 96.8%; specificity, 94.7%) makes it an ideal test for routine clinical practice.

But, at the same time, the absence of a direct and exclusive mechanistic link between HPV-DNA integration and p16 expression warns against a casual application of p16 testing alone. Westra (23) argues that p16 staining as standalone test for HPV detection is associated with a small false positive rate where p16 expression is driven by some non-viral mechanism. These p16 positive/HPV-negative oropharyngeal carcinomas have the same prognosis as the group of patients defined as HPV-negative OPSCC.

Therefore, the ideal tool for our study would be the use of a complementary test that detects mRNA, thus proving viral integration into the cell's DNA. Techniques that assure casualty remain economically challenging, and are not available in our Hospital. Due to the small rate of false positives, and the absence of therapeutic implications of our investigation, there is no justification for including a PCR or HIS determination in our study, as p16 remains a satisfactory technique for achieving our objectives.

Difficulties for data collection: When analyzing our results, an information bias must be considered, since a fair amount of sensitive information (about toxic and sexual habits) will be collected. Our investigators are trained to obtain this kind of data for clinical histories of patients, but we have to take into account the possibility of some of our participants hiding crucial epidemiological information when filling the data collection form.

An ethical limitation also determined the conception of the Data Collection Form, since the investigator's will was to collect information about the sexual orientation of our patients. A consultation was made to the CEIC, which determined the ethical impossibility to inquire that aspect.

Cross-sectional studies deal with an intrinsic limitation: they are not able to establish causal relationships, since it is impossible to set a temporal sequence.

Also, findings related to the covariates will require further study, since our primary objective deals merely with the relation between HPV and oropharynx cancer.

10. ETHICAL ASPECTS

The ethical principles established by the World Medical Association (WMA) in the *Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects* (June 1964, last review from October 2013) will be respected and serve us as an ethical guide for our investigation.

The first step towards the execution of the study is the submission of this protocol for approval by the Clinical Research Ethical Committee (CEIC, "*Comitè Ètic d'Investigació Clínica*") of the Hospital Universitari Dr. Josep Trueta.

Clinical and personal data from our patients will be confidential, and used exclusively for research purposes, according to the "*Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal*". Every piece of information will be analyzed anonymously through a numeric code system, in order to guarantee the confidentiality of the process.

Participants will be informed by their physician about the present study, and an information document (see *Annex 3*) will be handed to them. An informed consent (see *Annex 4*) will also be signed before entering the study, according to the "*Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica*".

No conflicts of interests are declared by the investigators in charge of this study.

11. BUDGET

The research team is composed by the Otorhinolaryngology department physicians, supposing no additional expense to the project. The whole *Quick Diagnosis* circuit is already consolidated and functioning, and the Head and Neck Functional Unit will suppose no special expense as well.

A statistic analyzer is needed, and considering the average fare, 525€ will be needed for this purpose. Each p16 inmunohistochemical determination costs an average of 40€, according to our Pathology laboratory.

The rest of the budget is composed by publication expenses.

EXPENSES		COST (€)
Personnel expenses		
Executive expenses		
	P16 determination (40€ x 51 patients)	2.040 €
	Statistical Analysis (15h per 35€/h)	525 €
	Consumable material	300 €
Publication expenses		
	Scientific publications	1.500 €
	Attendance to scientific meetings and congresses (SCORL, EAROL).	3.250 €
TOTAL		**7.615 €**

12. IMPACT OF THE PROJECT

As exposed in the Justification paragraph, we are in the threshold of a new approach to HPV-related oropharyngeal squamous cell carcinoma. Epidemiological information derived from our study will provide the multidisciplinary teams that treat these patients with new and relevant tools: A complete characterization of this type of patients will redound in a better understanding of the disease, thus enhancing the quality of the healthcare provided by doctors.

Additionally, what we hope that will follow this study is a systematic implementation of the p16 determination in every oropharyngeal squamous cell carcinoma in the province of Girona, since we think that the dissemination of our results among the scientific community will sensitize our local colleagues about this issue.

There is another interesting implication that would result from a systematic determination of HPV infection in our oncologic patients: Identifying HPV presence in a SCC in a neck lymph node strongly points to the oropharynx as the site of tumor origin, thus helping clinical management of early cancer detection.

Later on, our aim is that p16 determination through immunochemistry will be replaced by more specific techniques, such as PCR or HIS determination, as discussed in the Limitations paragraph. This will become especially relevant when the de-escalation treatment options are a reality.

13. ANNEXES

13.1 ANNEX 1: ANATOMY OF THE OROPHARYNX

The pharynx is a muscular tube that connects the nasal cavities to the larynx and esophagus. It is common to both the gastrointestinal and respiratory tracts. It is composed of three parts: the nasopharynx, oropharynx and laryngopharynx (see *Figure 8*).

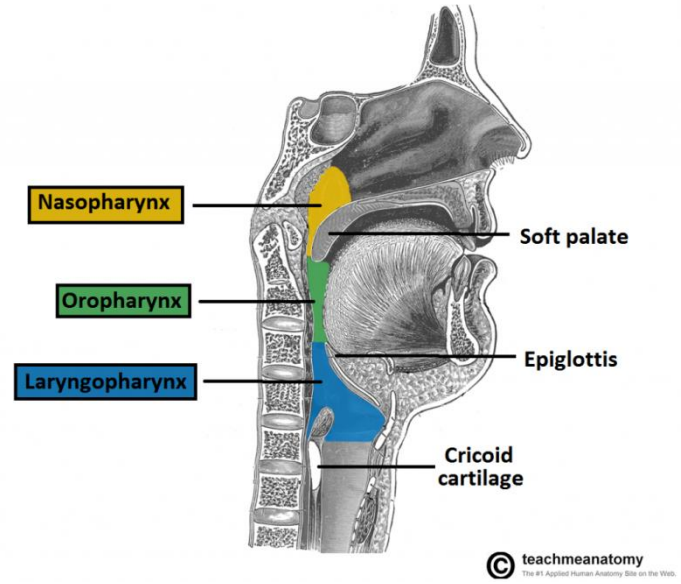

Figure 8: The three subdivisions of the pharynx and their borders (33)**.**

The oropharynx is the part of the throat just behind the mouth. In includes the base of the tongue, the soft palate, the tonsils and the side and back wall of the throat.

It is located behind the oral cavity (the palatoglossal arch) extending from the uvula to the level of the hyoid bone (34).

- It opens anteriorly into the mouth through the isthmus faucium. In this site, the oropharynx is delimited by the base of the tongue (posterior 1/3) and the upper border of the epiglottic vallecula.
- Laterally, it is formed by the palatine tonsils, tonsillar fossa, and tonsillar pillars located between the palatoglossal and palatopharyngeal arches.
- Superiorly, its wall consists of the interior surface of the soft palate and the uvula.

13.2 ANNEX 2: DATA COLLECTION FORM

Encuesta sobre variables epidemiológicas a estudiar en relación con la positividad para p16 en tumores de orofaringe

1. Código numérico asignado:
2. Fecha de nacimiento (día/mes/año): ___/___/_____
3. Sexo:
   - ☐ Hombre
   - ☐ Mujer
4. Etnia:
   - ☐ Caucásica
   - ☐ Latinoamericana
   - ☐ Africana
   - ☐ Asiática
5. Tabaquismo:
   - ☐ No fumador
   - ☐ Fumador (___cig/d)
   - ☐ Ex fumador
6. Alcohol:
   - ☐ No consumidor
   - ☐ Consumo moderado
   - ☐ Consumo elevado
7. Sexo oral:
   - ☐ Nunca
   - ☐ 2 parejas diferentes o menos
   - ☐ Más de 2 parejas diferentes
8. Localización de la neoplasia primaria:
   - ☐ Amígdala
   - ☐ Base de lengua
   - ☐ Úvula
   - ☐ Paladar blando
   - ☐ Pared faríngea posterior
   - ☐ Pared faríngea lateral.
9. Estadio T al comienzo del estudio:
   - ☐ Tis
   - ☐ T1
   - ☐ T2
   - ☐ T3
   - ☐ T4
10. Estadio N al comienzo del estudio:
    - ☐ N0
    - ☐ N1
    - ☐ N2a
    - ☐ N2b
    - ☐ N2c
    - ☐ N3
11. Metástasis al comienzo del estudio:
    - ☐ MX
    - ☐ M0
    - ☐ M1
12. Grado histológico tumoral al diagnóstico:
    - ☐ GX
    - ☐ G1
    - ☐ G2
    - ☐ G3
    - ☐ G4

13.3 ANNEX 3: INFORMATION DOCUMENT FOR THE PATIENT

INVESTIGADORES PRINCIPALES: _____

CÓDIGO DEL PROYECTO: _____

TÍTULO DEL ESTUDIO: **Nuevas tendencias epidemiológicas en el cáncer de orofaringe relacionado con Virus del Papiloma Humano en Girona: un estudio transversal**

El equipo de investigadores del Servicio de Otorrinolaringología del Hospital Universitario Josep Trueta de Girona propone la realización del estudio arriba mencionado, basado en observaciones propias y trabajos científicos de investigación médica.

1) Generalidades sobre el estudio

Está a punto de participar en un estudio llevado a cabo por el servicio de Otorrinolaringología del Hospital Universitario Dr. Josep Trueta, el cual tendrá una duración estimada de dos años. Se trata de un proyecto de investigación aprobado por el Comité Ético de Investigación Clínica del Hospital Dr. Josep Trueta. Los participantes en el mismo colaborarán mediante la aportación de datos pertenecientes a su historia clínica, los cuales serán recogidos en un formulario anónimo, y mediante la determinación de la expresión de un marcador inmunohistoquímico (p16) en la muestra de tejido recogido en su biopsia.

2) Objetivos del estudio

Se pretende conocer el porcentaje de pacientes con cáncer de orofaringe que están asimismo infectados por el virus del papiloma humano. De manera paralela pretendemos recoger datos epidemiológicos sobre cada caso que permitan caracterizar ambos tipos de cáncer (positivo y negativo para infección por el virus), a fin de contribuir al conocimiento teórico sobre esta patología, hecho que redundará en una mejora de la calidad asistencial que se proporciona a pacientes como usted.

3) Participación

Su participación en el estudio es voluntaria, siendo libre de decidir si sus datos serán incluidos en nuestro análisis o no. Puede negarse a hacerlo sin necesidad de justificación, y sin que esto afecte a la calidad de la asistencia sanitaria que reciba. La participación en el estudio es gratuita, y no obtendrá compensación económica por su participación.

¿Por qué es beneficiosa su participación en el estudio?

Nuestro interés es analizar datos obtenidos de su historia clínica y de la biopsia de su lesión. Así pues, participar en el estudio no le supone la realización de ningún procedimiento complementario a los que se habrían de realizar en caso de no dar su consentimiento. Tan sólo habrá de rellenar, con ayuda de su médico, un cuestionario que recoge datos sobre diversos aspectos de su historia clínica.

4) Criterios de exclusión. Usted no puede participar en el estudio si:

- Fue diagnosticado de cáncer de orofaringe con anterioridad al inicio de este estudio.
- Padeció alguna otra neoplasia en orofaringe.
- Está contraindicada en usted la toma de una biopsia.

5) Confidencialidad y protección de datos

Bajo el cumplimiento de la *Ley Orgánica* 15/1999 se adoptarán las medidas pertinentes para garantizar la confidencialidad de sus datos. La información recogida será gestionada de manera anónima (utilizando un código numérico), y únicamente utilizada con fines de investigación. Se garantizará el cumplimiento de los principios establecidos por la *Ley de Investigación Biomédica* 17/2007.

6) Funciones del participante en el estudio

Junto al médico, el participante deberá rellenar la encuesta proporcionada en el Anexo 3. La determinación del p16 mediante inmunohistoquímica será realizada por el equipo de Anatomía Patológica del Hospital Universitario Josep Trueta sobre el tejido extraído en la biopsia al paciente.

7) Resultados del estudio y beneficios

El paciente está en su derecho de ser informado de los resultados el estudio, o de mantenerse al margen. Los beneficios derivados de la información analizada por el equipo de investigación, pueden verse reflejados en el paciente o en otras personas. Los frutos del trabajo de investigación estarán al servicio de cumplir con los objetivos del estudio, y servirán como base a futuras investigaciones en dicho ámbito de conocimiento.

Gracias por su participación.

Información de contacto:

Alejandro Pérez Rizo (colaborador del equipo de investigación)

Dirección: C/ Emili Grahit, 77, 17071 Girona

Teléfono de contacto: 670423577 (de 9:00 a 15:00h)

Correo electrónico: aperezrizo92@gmail.com

13.4 ANNEX 4: INFORMED CONSENT

Declaración del participante:

Yo,_____, con documento de identidad _____ certifico que:

- He leído la hoja informativa sobre el estudio.
- He tenido oportunidad de hacer todas las preguntas necesarias respecto al estudio.
- Estoy conforme en relación a la cantidad de información recibida.
- He sido informado por el investigador _____ sobre las implicaciones y finalidades del estudio.
- Entiendo que mi participación es voluntaria.
- Entiendo que mis datos y pruebas serán etiquetados con un código numérico a fin de mantener la confidencialidad.
- Entiendo que, según la Ley de Biomedicina 14/2007 de investigación Biomédica, el material sobrante del estudio será utilizado en futuros proyectos relacionados con éste, o bien destruido, según mi voluntad.
- Entiendo que puedo, conforme a la "*Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica",* revocar el consentimiento de participación en el estudio, sin tener que justificarlo, y sin que ello afecte a mi asistencia sanitaria.

Acepto que los investigadores principales del proyecto puedan contactarme en el futuro si se considera oportuno:

☐ Sí ☐ No

Libremente doy mi conformidad para participar en el estudio aportando datos de mi historia clínica, y de la biopsia realizada:

☐ Sí ☐ No

Autorizo a que futuros estudios similares a este utilicen los datos de mi historia clínica, como fuente de datos epidemiológicos:

☐ Sí ☐ No

Firma del participante Firma del investigador

Fecha: __/__/____ Fecha: __/__/____

APARTADO PARA LA REVOCACIÓN DEL CONSENTIMIENTO

Yo,_____, revoco el consentimiento de participación al estudio.

Fecha: __/__/____ Firma