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DOCTORAL SCHOOL
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***VIRAL INVOLVEMENT IN ONCOGENESIS AND THE
MANAGEMENT OF CANCERS OF THE UPPER
AERODIGESTIVE TRACT***

ABSTRACT OF THE DOCTORAL THESIS

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List of papers published in specialist journals:

1. Viral involvement in the oncogenesis of oropharyngeal squamous cell carcinoma: from molecular mechanisms to therapeutic strategies

Dan-Alexandru Popescu, Miruna-Maria Apetroaei-Leucă, Mihai-Dumitru Tudosie, Simona-Andreea Rujan, Bianca-Petra Taher, Raluca Grigore, Alexandra Maria Marin, Gloria-Simona Berteșteanu, Diana Cosmina Drăgan, Bogdan Popescu, Șerban Vifor-Gabriel Berteșteanu, *Farmacia* 2026; 75.

2. Multi-Step Surgical Management in a Patient with Recurrent Laryngeal Papillomatosis

Dan-Alexandru Popescu, Mihai-Dumitru Tudosie, Simona-Andreea Rujan, Bianca-Petra Taher, Raluca Grigore, Alexandra Maria Marin, Gloria-Simona Berteșteanu, Diana Cosmina Drăgan, Bogdan Popescu, Irina Doinița Popescu, Șerban Vifor-Gabriel Berteșteanu, *Journal of Medicine and Life* 2026.

3. The Impact of HPV Infection on the Development of Oropharyngeal Cancer: A Single-Centre Experience

Dan-Alexandru Popescu, Mihai-Dumitru Tudosie, Simona-Andreea Rujan, Bianca-Petra Taher, Raluca Grigore, Alexandra Maria Marin, Gloria-Simona Berteșteanu, Diana Cosmina Drăgan, Bogdan Popescu, Irina Doinița Popescu, Șerban Vifor-Gabriel Berteșteanu, *Surgery*.

Introduction

In 2022, according to the National Institute of Public Health, Romania had a cervical cancer incidence rate of 32.6 new cases diagnosed annually per 100,000 women and a standardised mortality rate approximately three times higher than the European Union average. With a National Screening Programme for this type of cancer associated with HPV infection, the inclusion of vaccination against the virus in the National Immunisation Programme (PNV) and the extension of free vaccination to those aged between 11 and 26, our country is striving to meet the target set by the World Health Organisation of reducing the incidence of the disease to 4 cases per 100,000 women by 2030.

At the same time, another cancer associated with Human Papillomavirus (HPV) infection is seeing a sharp rise in incidence, particularly in developed countries. HPV-positive oropharyngeal cancer was defined by the AJCC (American Joint Committee on Cancer) as a distinct pathological entity from 2018 onwards, when its staging underwent changes compared with HPV-negative oropharyngeal cancer. This change was necessary due to the distinct aetiopathogenesis and prognosis of the disease, which is better than that of HPV-negative oropharyngeal cancer – conclusions supported by research over the last few decades. In Romania, the rate of increase in the incidence of this disease is lower than in many other countries, but when considering oropharyngeal cancer of any aetiology, we rank among the highest in Europe, with approximately 11 new cases diagnosed annually per 100,000 men.

Cancer remains the second leading cause of morbidity and mortality worldwide, after circulatory system diseases (Eurostat, 2026) .

Often diagnosed at advanced stages of the disease, cancer of the upper aerodigestive tract is a debilitating condition due to its potential to affect a vital bodily function (breathing, via an obstructive mechanism) and two other related functions: swallowing and speech. To maintain an acceptable quality of life following treatment, the approach to this condition is individualised, depending directly on the location, extent of the tumour and the stage of the disease. In recent years, surgical treatment has benefited from the advent of minimally invasive surgery, which is currently gaining ground in our country too, particularly in university hospitals. Suspended micro-laryngoscopy using a CO₂ laser is an increasingly common treatment option in the early stages of the disease.

One of the reasons for choosing this topic was the need for primary prevention of neoplastic diseases in the ENT field, which, unlike other cancers, is on the rise in incidence statistics. The lack of adequate health education, socio-economic conditions, as well as family breakdown, are factors which, in many cases lead to patients delaying their visit to the doctor, late diagnosis and a poor prognosis.

The implementation of a National Screening Programme for the early detection of (pre)cancerous lesions in the oropharynx, as well as the creation of a centralised database, would bring significant benefits to the survival of these patients and would enable specialist medical staff to monitor and manage the condition more effectively.

This study involved a retrospective analysis of a homogeneous group of patients treated at a tertiary centre and spanned a period of 3 years (2020–2022). The collected data set was statistically analysed using the R programme, version 4.6.0, (c) 2026 The R Foundation for Statistical Computing.

The first study aimed to describe the patient profile by analysing clinical and epidemiological characteristics, as well as tumour characteristics, and to identify factors associated with the severity of the neoplasm, which was quantified using overall survival (OS).

The second study aimed to assess the impact of biological and therapeutic factors on disease progression and prognosis, and to determine the 12-month, 24-month and 60-month RMST in close correlation with the treatment administered.

I. Current state of knowledge

1. Viruses with oncogenic potential

In developing and drafting the chosen topic, ‘Viral involvement in oncogenesis and the management of cancers of the upper aerodigestive tract’, we took as our starting point the current state of knowledge in the field, which is constantly evolving. According to the World Health Organisation (WHO), approximately 20 per cent of all cancers worldwide are associated with infectious agents. When considering specific viruses, there are seven known to be precursors to the development of cancers :

1. Epstein-Barr virus,
2. Hepatitis B virus,
3. Human T-lymphotropic virus type 1,
4. Human papillomavirus,
5. Hepatitis C virus,
6. Kaposi’s sarcoma-associated herpesvirus,
7. Merkel cell polyomavirus.

As the human immunodeficiency virus (HIV) causes cancer only by potentiating the carcinogenic action of other infectious agents, it has not been considered separately; neoplasms occurring in HIV-positive patients are attributed to co-infection (IARC) (Krump NA, Molecular mechanisms of viral oncogenesis in humans, 2018) .

As this is a topic of public health interest, I considered it appropriate to focus my attention on the human papillomavirus and HPV-positive oropharyngeal cancer, primarily due to the rising incidence of this type of ENT neoplasm, but also due to the prevalence of HPV.

1.1. A brief history

In 1942, Parsons and Kidd described the areas of the oral mucosa most prone to infection with the Papilloma Virus and demonstrated that even local irritation or trauma can activate a latent Papilloma Virus infection (Wojciech Golusiński, 2017) .

In the second half of the 20th century, Peyton Rous was awarded the Nobel Prize for his discovery of the role of viruses in oncogenesis (1996) (Morhaf Jalab) .

Furthermore, in 1989, Brandsma and Abramson identified viral DNA (subtype 16) in squamous cell carcinoma of the tonsil (Wojciech Golusiński, 2017) , and after the 2000s, research in this field intensified, partly due to the increasing incidence of associated pathology.

1.2. Carcinogenesis and neoplasms of the upper aerodigestive tract

Cancer is an abnormal growth of cells resulting from changes in gene expression, which lead to an imbalance between cell proliferation and cell death, ultimately developing into a population of cells that invade tissues, metastasise to distant sites and can cause the death of the host in the absence of appropriate treatment (Morhaf Jalab) .

Proto-oncogenes are genes involved in the four basic regulators of cell growth: growth factors, growth factor receptors, nuclear transcription factors and signal transduction molecules (Morhaf Jalab) . When a mutation occurs in a proto-oncogene, it can become an oncogene, with a single copy being sufficient for tumour progression. Tumour suppressor genes suppress tumour formation by controlling cell growth, and they encode proteins whose absence, inactivation or mutation promotes oncogenesis (Morhaf Jalab) . The presence of viral DNA in tumour cells (the entire viral genome or just part of it) attests to the virus's oncogenic potential (Morhaf Jalab) .

Squamous cell carcinomas of the head and neck are the 7th most common neoplasm, with 890,000 new cases and 450,000 deaths annually (according to GLOBOCAN estimates) (Barsouk A, 2023) . These tumours develop in the mucosa of the upper aerodigestive tract (oral cavity, nasopharynx, oropharynx, hypopharynx, larynx and salivary glands), which play an important role in breathing, swallowing and speech. Human Papillomavirus (HPV)

HPV is a double-stranded DNA virus. The viral genome is organised into three regions: the early gene region (E), the late gene region (L) and the long control region (SV, 2017) . The viral oncogenes E6 and E7 encode oncoproteins that promote viral replication. E6 binds to and inactivates the tumour suppressor protein p53, which is responsible for cellular apoptosis. E7 binds to and inactivates the Rb protein, releasing the E2F transcription factor, which activates DNA synthesis by promoting the transcription of genes involved in this process (Cam H, 2003) (Flores ER, 2000) .

The p16 protein is directly involved in regulating the cell cycle as a compensatory mechanism following the inactivation of the pRb protein. It can be detected by

immunohistochemistry and is present in over 70% of tumour cells. HPV testing using p16 immunohistochemistry should be carried out in all patients diagnosed with oropharyngeal squamous cell carcinoma, as p16 is a valid surrogate marker for HPV positivity, even though false-positive results may occur in approximately 10–15% of cases (Machiels JP, 2020)

1.3. The natural history of oral HPV infection

The main route of HPV transmission is sexual contact (skin-to-skin or skin-to-mucous membrane) (Wierzbicka M, 2023) (Petca A, 2020) . There is little evidence regarding transmission via saliva or other routine interactions; oral transmission of HPV infection is primarily linked to sexual contact (Wierzbicka M, 2023) . However, other routes of transmission are also possible, such as autoinoculation, indirect transmission via the hands, vertical transmission from mother to child during direct contact with the genital tract of the infected mother, or via ascending infection following premature rupture of the membranes (Petca A, 2020) . There is a latency period between exposure to HPV, infection, chronic infection and cancer, which can last 10 years or even 2–3 decades (Taberna M, 2017) . In most cases, the immune system clears the virus within 7 months to 2 years of infection, but when this does not happen, the risk of developing a precancerous lesion increases. If the precancerous lesion does not regress, it may progress to invasive cancer (Taberna M, 2017) . HPV type 16 has the lowest clearance rate.

The life cycle of HPV is closely linked to the differentiation process of the host epithelium. The cryptic epithelium covering the tonsils and the base of the tongue acts as a reservoir for the virus, providing access to its basal layer for viral replication.

2. HPV-positive oropharyngeal cancer

2.1. Tropism of the Human Papillomavirus (HPV)

HPV has a predilection for the squamous epithelium of the tonsils and the base of the tongue (Hans Behrbohm, 2009) . There are two explanations for this localisation: both are part of Waldeyer's lymphatic ring, which is involved in the immune response, and the presence of crypts in the mucosa, which provide easier access to the only epithelial cells that divide, those in the basal layer.

The HPV16 strain is overwhelmingly predominant in the aetiology of oropharyngeal cancer, accounting for 87% of oropharyngeal squamous cell carcinomas (Goon P, 2022) (Bray F, 2017) (Buell, 1974) . There are also other positive associations, albeit at lower frequencies, for HPV types 18, 31, 33 and 52 (Goon P, 2022) (Michaud DS, 2014) .

P16INK4a is a tumour suppressor protein marker whose expression improves survival in oropharyngeal squamous cell carcinomas (Sedghizadeh PP, 2016) . The combined use of p16 and Ki-67 staining increases diagnostic accuracy, particularly in distinguishing low-grade from high-grade lesions (Bean SM, 2007) (Chen CC, 2020) (Watts AE, 2006) .

2.2. Risk factors for oropharyngeal cancer

The profile of a patient with HPV-positive oropharyngeal cancer is: male, high socio-economic status, non-smoker, non-drinker, reports a high number of sexual partners and engages in oral sex, has a history of drug use, and is of middle age (bimodal distribution across age groups: 30–50 years and 60–70 years).

2.3. Incidence of HPV-positive oropharyngeal cancer

In 2022, Romania had one of the highest rates of oropharyngeal cancer among men in the world, with 10.6 new cases per 100,000 men per year (Rumgay H, 2026) .

At least over the last two decades, there has been a sharp rise in the incidence of HPV-positive oropharyngeal cancers, which in some developed countries have now overtaken HPV-negative cases.

2.4. The prevalence of HPV in oropharyngeal cancer

Epidemiological data suggest that the prevalence of HPV-positive oropharyngeal cancer may vary depending on a country's socio-economic status (Lu Y, 2022) .

When referring solely to the prevalence of oral HPV infection, the study published in 2012 shows a prevalence of 3%, including among people who report not engaging in oral sex, a percentage that doubles for those with 2–5 partners, rises to 10% for those with 6–10 partners, and reaches up to 22% for those with 21 or more partners (Gillison ML, 2012) .

2.5. Prevention methods

The first line of defence is (Timbang MR, 2019) :

- a. Vaccination!
- b. Screening methods are becoming increasingly important and necessary for increasing life expectancy.
- c. Lifestyle changes, through the promotion of safe sexual practices.
- d. The technique for detecting viral DNA in saliva is of limited effectiveness, as in most cases HPV infection does not progress to malignancy.
- e. HPV serology to detect antibodies (against HPV16 proteins) may prove useful in identifying at-risk populations.
- f. The most reliable method for identifying early-stage lesions is NBI (narrow-band imaging), which has much higher sensitivity and accuracy than white-light fibroscopy.

2.6. TNM staging

Stage I	T1 tumour $\leq$ 2 cm. T2 tumour $>$ 2 cm and $\leq$ 4 cm.
T1N0M0/ T2N0M0	No lymph node involvement and/or metastases.
T0N1M0, T1N1M0/ T2N1M0	Primary tumour undetectable or smaller than 4 cm. Ipsilateral lateral cervical lymph node involvement, lymph nodes $\leq$ 6 cm.
Stage II	The primary tumour is not found or is $\leq$ 4 cm.
T0N2M0/ T1N2M0/ T2N2M0	Contralateral lateral cervical lymph node involvement, lymph nodes $\leq$ 6 cm.
T3,N0,M0 T3N1M0/ T3N2M0	T3 tumour $>$ 4 cm or with invasion of the epiglottis. Lateral cervical lymph node involvement, lymph nodes $\leq$ 6 cm.
Stage III	Primary tumour not detectable (T0)

T0N3M0/ T1N3M0, T2N3M0/ T3N3M0	If found, it is of any size or invades the epiglottis (T1, T2, T3). Lateral cervical lymph node involvement, at least one > 6 cm (N3).
T4N0M0/ T4N1M0 T4N2M0/ T4N3M0	A T4 tumour invades the larynx, the tongue muscle, the bones of the oral cavity (medial pterygoid, hard palate, maxilla) or extends further into the neck or head. Lymph node involvement on the left side.
Stage IV	
Any T, Any N, M1	Distant tumour invasion from the oropharynx (M1)

(Zanoni DK, 2019)

Disease staging has undergone changes since 2018 with regard to HPV-negative oropharyngeal cancer. Thus, for ipsilateral lateral cervical lymph nodes with a diameter of less than 6 cm, only N1 staging remains; for lymph nodes located contralaterally or bilaterally, with a size of less than 6 cm, we have N2 staging; and stages T4a and T4b have been merged into T4, due to similar patient survival rates.

2.7. treatment

Stage I (N0) No lymph node involvement	2 treatment options: Radiotherapy, followed by imaging follow-up Surgery ± adjuvant therapy
Stage I (N1) Lymph node involvement with 1 lymph node ≤ 3 cm	3 treatment options: Radiotherapy, followed by imaging follow-up Surgery ± adjuvant therapy RT + chemotherapy, followed by imaging follow-up
Stage I (N1) Lymph node involvement with 1 lymph node > 3 cm or the presence of at least 2 lymph nodes Stage II Stage III	2 treatment options: Radiotherapy + chemotherapy, followed by imaging follow-up Surgery and adjuvant therapy
Stage IV	Chemotherapy. Palliative care

AJCC^{8th} Edition (Zanoni DK, 2019)

2.8. Side effects of treatment:

- a. **Surgical:** aphonia, speech difficulties, difficulty swallowing, ear paraesthesia, cosmetic issues, thyroid hormone deficiency (hypothyroidism), local pain, local infection.
- b. Some common side effects of **radiotherapy** are as follows: fatigue, skin redness, dryness and itching, skin peeling, xerostomia, altered taste, odynophagia.
- c. The most common side effects of **chemotherapy** are: nausea/vomiting, hypoaesthesia of the fingers, renal impairment, hearing loss, tinnitus, eczema, and an increased risk of infections associated with radiotherapy.

2.9. New trends in treatment strategy – De-escalation of therapy

- Patients with HPV-positive oropharyngeal cancer generally have a lower average age.
- The side effects of treatment have clinical and socio-economic implications.
- Patients with HPV-positive oropharyngeal cancer have a favourable prognosis.

All these factors have led to interest in de-escalation of therapy, with the aim of maintaining survival rates whilst reducing acute and late toxicity resulting from treatment.

2.10. Comparative prognosis (HPV-positive OPC vs HPV-negative OPC)

The risk of death decreases by 28%, and the risk of recurrence by 49% in patients diagnosed and treated for HPV-positive OPCs, compared with those with HPV-negative OPCs (Berman TA, 2017). **Why do we have a favourable prognosis?** (Berman TA, 2017)

- a) In HPV-positive cancers, secondary inactivation of the p53 protein occurs without any alteration to the gene. Chemotherapy or radiotherapy may enhance the anti-tumour activity of this protein.
- b) Patients diagnosed with HPV-positive cancer are generally younger and have fewer comorbidities.
- c) Genetic factors are particularly involved in HPV-negative tumours.
- d) Persistent HPV in the tonsils may trigger an anti-tumour immune response to specific viral antigens following chemotherapy or radiotherapy.
- e) HPV-positive tumours are more radiosensitive.
- f) HPV-positive tumours may be detected at an early stage due to the onset of laterocervical lymphadenopathy, which alerts the patient and prompts them to seek medical attention.
- g) HPV-negative OPCs exhibit locoregional spread and are more resistant to treatment.

I. PERSONAL CONTRIBUTIONS

3. Working hypothesis and general objectives

3.1. Working hypothesis

The present study is based on the hypothesis documented in the specialist literature regarding the decisive role of oncogenic viral agents – in this case, the Human Papillomavirus – in the aetiopathogenesis of squamous cell carcinomas of the upper aerodigestive tract, influencing the clinical course of the disease. The increase in the incidence of HPV-positive oropharyngeal cancers has also been a reality over the last two decades; in some Western countries, their number has exceeded that of HPV-negative oropharyngeal cancers.

Assessing the HPV status of tumour proliferation is of particular importance, as a positive result represents a favourable prognostic factor, associated with a lower risk of death and/or recurrence.

Squamous cell carcinomas of the head and neck are the seventh most common neoplasm, with nearly 900,000 new cases diagnosed and 450,000 deaths annually (according to GLOBOCAN estimates) (Barsouk A, 2023) (POPESCU Dan. A.-L. M., 2026)

The present study aims to analyse 67 inpatients diagnosed with cancers of the upper aerodigestive tract and treated in both the ENT department and the Radiotherapy and Medical Oncology departments at Colțea Clinical Hospital, Bucharest. Data were extracted through the analysis of medical records (physical or electronic medical records, histopathology reports, immunohistochemistry reports and surgical protocols).

3.2. Research objectives

General objectives:

- To update information relating to the chosen topic;
- To conduct a comparative analysis of the data obtained from the study forming the subject of this PhD thesis with existing data in the specialist literature;
- To obtain statistically significant results following the analysis of the case studies included in the study; these results will confirm or refute the working hypothesis and are intended to serve as a reference point for future research.

Immediate objectives:

- Selective identification of patients' demographic factors,
- Analysis of medical investigations,
- Analysis of treatment protocols,
- Final staging, following completion of the HP examination with immunohistochemistry tests, in the context of the 'downstaging' phenomenon observed in HPV-positive oropharyngeal carcinoma, as formalised in the cancer staging manual (AJCC, 2018).
- Assessment of response to unimodal/multimodal therapy.

4. Research methodology

This paper is structured around two studies conducted over a three-year period (from 2020 to 2022 inclusive).

Inclusion criteria for the study:

- patients aged over 18;
- patients who have signed the informed consent form;
- patients who presented at Colțea Clinical Hospital, Bucharest, either at the ENT Specialist Outpatient Clinic, the A&E Department, or the ENT ward;
- patients clinically assessed and diagnosed with tumours of the upper aerodigestive tract;
- patients diagnosed, following a tumour biopsy, with cancers of the upper aerodigestive tract based on histopathological examination of the tissue;
- patients who underwent IHC to assess the HPV status of tumour proliferation.

Exclusion criteria from the study:

- patients under the age of 18;
- patients with incomplete data;
- patients in whom histopathological examination ruled out the malignant nature of the tumour;
- patients who, following tumour biopsy, underwent treatment at other medical centres, could not be assessed over time, and for whom we found no information regarding their survival.

Variables studied:

- distribution by gender;
- distribution by age group;
- risk factors;
- comorbidities;
- tumour location;
- clinical diagnosis;
- histopathological diagnosis;
- immunohistochemical diagnosis;
- disease staging;

- paraclinical investigations;
- biological parameters;
- surgical treatment;
- adjuvant therapy;
- follow-up;
- recurrence rate;
- survival rate;

The motivation behind the choice of this research topic is also driven by the need to increase the HPV vaccination rate. At the current rate, it will take Romania at least 50 years to reach the HPV immunisation rate recommended by the World Health Organisation (90%). In 2024, the vaccination rate in Romania stood at just 7.5 per cent, a figure that places us among the lowest-ranked countries in the European Union.

The R programme, version 4.6.0, (c) 2026 The R Foundation for Statistical Computing, was used for the statistical analysis. We employed both descriptive statistical analysis and inferential statistics (Kaplan-Meier analysis, Cox regression, log-rank test, ROC analysis).

The database is shared by both studies and is organised by calendar year as follows:

- in 2020 – 34 patients,
- in 2021 – 19 patients,
- in 2022 – 14 patients.

5. Study I

Clinical, epidemiological and anatomopathological analysis of patients with squamous cell carcinoma of the upper aerodigestive tract

5.1. Introduction

A retrospective, non-randomised observational study involving 67 cases. The study population consisted of 67 patients diagnosed with neoplasms of the upper aerodigestive tract who underwent oncological treatment; this sample is representative of a population of patients with neoplasms of the upper aerodigestive tract, diagnosed and treated at a tertiary centre.

We used descriptive statistics for demographic characteristics (age, sex), risk factors (tobacco, alcohol, occupational hazards), personal medical history, clinical presentation (symptoms and clinical signs), the distribution of tumour sites, disease stage and tumour differentiation grade.

The main objectives of Study I were:

- to describe the profile of the patients included, by analysing their clinical and epidemiological characteristics, as well as tumour characteristics,
- to identify factors associated with tumour severity, which was quantified using overall survival (OS).

Complete data were not available for all patients; no imputation method was used to recover these data.

5.2. Materials and methods

The R programme, version 4.6.0, (c) 2026 The R Foundation for Statistical Computing, was used for statistical analysis.

The primary endpoints of the study were death (yes/no) and survival time (months).

The study's alpha significance level was 0.05, with p-values less than 0.05 considered statistically significant.

A combined survival analysis was used: for the independent variables—which comprised various demographic, clinical and paraclinical categorical parameters—a Kaplan-Meier analysis and a log-rank test were used, whilst for the continuous variables, Cox regression was used; subsequently, a cut-off value for the variable was determined using ROC analysis, and based on this value, two categories of the independent variable were created, which were analysed in accordance with the protocol mentioned for categorical variables.

5.3. Results and discussion

The gender distribution of cases in the study cohort favoured men, with a ratio of 8.5:1. Data from the specialist literature indicate a ratio of 3.5:1, and if we consider oropharyngeal cancer specifically, a ratio of 5:1 (Dahlstrom KR, 2011) .

The course of the disease was more severe in males, but the differences were not statistically significant.

The patients' ages ranged from 38 to 78 years, with a mean of 60 years and a median of 62 years. The specialist literature indicates the highest incidence in the sixth and seventh decades of life, respectively (Dahlstrom KR, 2011) . Currently, with regard to HPV-positive oropharyngeal cancer, we can observe a bimodal distribution across age groups, namely 30–50 years and 60–70 years.

The course of the disease was more severe in smokers, but the difference was not statistically significant.

Clinical symptoms were dominated by dysphagia and odynophagia, with most patients presenting with at least two subjective complaints simultaneously.

With regard to personal medical history, the vast majority of patients had associated cardiac conditions, predominantly hypertension (35.8%), but also tuberculosis (12%), a second neoplasm (9%), COPD (7.5%) or diabetes mellitus (6%).

The medical investigations undertaken on patients in the study cohort included clinical, biological, paraclinical and imaging tests, which were generally readily available, with the exception of PET-CT, due to high costs.

85 per cent of the cases included in the study were localised in the oropharynx. Of these, 26.5 per cent were HPV-positive cancers, whilst 73.5 per cent were HPV-negative cancers. The

specialist literature indicates that in some Western countries, the number of HPV-positive oropharyngeal cancers has exceeded that of HPV-negative oropharyngeal cancers.

Distribution of cases by tumour stage: 5 patients (representing a total of 7.5%) were diagnosed at stage I of the disease, 11 patients (16.5%) were diagnosed at stage II of the disease, 16 patients (24%) were diagnosed at stage III, 20 patients (30%) were diagnosed at stage IVa, 11 patients (16.5%) were diagnosed at stage IVb and 2 patients (3%) were diagnosed at stage IVc. We note a predominance of patients diagnosed at advanced stages of the disease, which will also be reflected in the overall survival rate.

The most common grade of tumour differentiation in the cohort of cases studied was well-differentiated G1 squamous cell carcinoma (27 cases, accounting for 40% of all cases).

There were significant differences in clinical outcome, with T1 and T2 tumours showing a much better prognosis than T4 tumours. For early-stage T tumours, the median 12-month survival was more than 2 months longer, the median 24-month survival was almost 8 months longer, and the median 60-month survival was more than 30 months longer.

The prognosis was much better for patients with N0 and N1 disease. For early-stage N disease, the median 12-month survival was more than 2 months longer, the median 24-month survival was almost 8 months longer, and the median 60-month survival was more than 29 months longer.

In the early stages of the disease (I and II), the median 5-year survival was more than 30 months longer than in the more advanced stages.

Immediately after documenting the positive diagnosis confirming malignancy (following review of the histopathological examination reports), we found that the vast majority of patients (over 70%) had not undergone immunohistochemical testing to determine the HPV status of the tumour. The reasons cited included: restrictions during the Covid-19 pandemic, the absence of carers or social support, financial difficulties, and one and logistical challenges. Consequently, we submitted an official request to carry out retrospective immunohistochemistry tests for the vast majority of patients included in the study. Following the approval of the request, with the collaboration of Dr Ileana Popa, Dr Luminița Welt (Head of the Department of Pathological Anatomy) and the entire medical team, both the p16 biomarker and the Ki-67 cell proliferation biomarker were analysed in 40 of the cases included in the study.

In the p16 analysis, the differences were statistically significant: p16-positive => better prognosis. After confirming the presence of HPV infection in tumour cells by assessing p16

protein expression via immunohistochemistry, we re-staged the cases in accordance with the AJCC Cancer Staging Manual, 8th edition. In 2018, the staging of HPV-associated oropharyngeal cancer underwent changes, due to the better response to treatment and the favourable prognosis that these patients have compared with forms caused by other aetiological factors (smoking, alcohol, etc.).

Age and Ki67 expression percentage are continuous variables; we used a simple Cox regression to investigate their effects on OS and found a positive association between the Ki67 expression percentage and the hazard of death: a one-unit increase is associated with a 2% increase in the hazard of death.

We used a survival ROC analysis and calculated a cut-off value for Ki67 expression; the value tested was 80% Ki67 expression. We used this cut-off value to divide the patients into two categories. Although the differences are at the borderline of statistical significance, there is evidence that in patients with Ki67 expression levels above 80%, the disease course is more severe (the analysis was performed only on a subsample of 54 patients with complete Ki67 data).

In an analysis model incorporating TNM staging and Ki-67 category, we observe that the influence of the Ki-67 category is stronger in the multiple model; a Ki-67 category of over 80% is less common in early-stage cancer.

6. Study II

Analysis of treatment and prognostic factors affecting survival

6.1. Introduction

An observational, retrospective, non-randomised study involving the same sample of 67 patients.

The main objectives of Study II were:

- to assess the impact of biological factors (the p16 marker) and therapeutic factors (surgery, radiotherapy, chemotherapy) on disease progression and prognosis.
- Determination of the RMST at 12, 24 and 60 months, in close correlation with the treatment administered.

Complete data were not available for all patients; no imputation method was used to recover these data.

6.2. Materials and methods

The R programme, version 4.6.0, (c) 2026 The R Foundation for Statistical Computing, was used for statistical analysis.

The key endpoints of the study were death (yes/no) and survival time (months).

The study's alpha significance level was 0.05, with p-values less than 0.05 considered statistically significant.

We used inferential statistics, specifically a combined survival analysis: for categorical variables, we used a Kaplan-Meier analysis and a log-rank test, whilst for continuous variables we used simple and multiple Cox regression models.

6.3. Results and discussion

Monotherapy was the treatment option in 17 (25%) of the cases: 3 patients received surgery alone, 8 patients received radiotherapy alone and 6 patients underwent palliative chemotherapy only.

Combination therapy was the treatment option in 50 (75%) of the cases: 17 patients underwent surgery and radiotherapy, 16 patients received e cancer treatment (radiotherapy and chemotherapy), 16 patients underwent surgery, followed by oncological treatment

(radiotherapy and chemotherapy), and 1 patient underwent surgery and chemotherapy. In all cases treated at Colțea Clinical Hospital, the treatment decision was made following interdisciplinary collaboration between ENT, oncology, radiotherapy, radiology and imaging, and pathological anatomy within the Oncology Committee.

It should be noted that, in very few cases, cancer treatment was influenced by the HPV status of tumour proliferation, given patients' non-compliance with medical recommendations regarding the need to supplement investigations with immunohistochemistry tests.

The main surgical procedures and techniques used:

- Trotter-type lateral pharyngectomy,
- Transjugular oropharyngectomy,
- Ablation of parapharyngeal tumour,
- Partial glossectomy with extended transmandibular oropharyngectomy,
- Transmandibular hemiglossectomy and palatine tonsil tumour resection,
- Huet hyoepiglottectomy extended to the base of the tongue,
- Transoral buccopharyngectomy with resection of the base of the tongue,
- Buccopharyngectomy via a combined approach (transjugular and lateral pharyngectomy),
- ¼ hemilaryngectomy with partial lateral pharyngectomy,
- Oropharyngectomy via a transcervical approach,
- Transmandibular lateral buccopharyngectomy,
- CO₂ laser tonsillectomy,
- Total suprahyoid, subcricoid laryngectomy with near-total glossectomy,
- Extended hyoepiglottectomy at the base of the tongue with laryngeal anchoring to the base of the tongue,
- Lateral transoral CO₂ laser buccopharyngectomy with partial glossectomy,
- Lateral buccopharyngectomy with resection of the intermaxillary commissure,
- Total uvulectomy.

The main procedures were supplemented by functional/radically modified ipsilateral/bilateral jugulo-carotid lymph node dissection, arterial ligations/resections, accessory spinal nerve resection, and submaxillectomy.

The study included 12 patients who required reconstructive surgery to repair the defect following radical surgery; 9 patients underwent emergency tracheotomy (representing 24.5 per cent), whilst 28 patients underwent elective tracheotomy (75.5 per cent). Furthermore, 46 patients had a nasogastric tube inserted, 5 patients had a feeding gastrostomy tube inserted, and 34 patients underwent both tracheostomy and feeding via a nasogastric tube or gastrostomy tube. The main techniques used for reconstruction were:

- Reconstruction of the lateral wall of the oropharynx and the floor of the mouth using a musculocutaneous flap from the sternocleidomastoid muscle,
- Mandibular osteosynthesis using a plate and screws,
- Reconstruction of a submandibular defect using a myofascial-cutaneous flap from the SCM muscle and recalibration of the tracheostomy stoma,
- Reconstruction of a jugulo-carotid fossa defect using a muscle flap from the sternocleidomastoid muscle.

The vast majority of patients underwent radiotherapy at the Radiotherapy Centre within Colțea Clinical Hospital. The total radiation dose prescribed ranged from 62–70 Gy per target volume at the tumour bed, usually divided into 33–38 fractions, and a dose of 20–50 Gy prescribed to the lymph node regions, usually divided into 10–25 fractions. In some selected cases, radiotherapy was initiated concurrently with cisplatin administration.

The treatment was generally well tolerated; the most common adverse reactions included: skin rash, post-radiation erythroderma, post-radiation oedema, post-radiation stomatitis, radiomucositis and radiodermatitis.

As regards chemotherapy, a significant proportion of the patients who underwent this treatment received it in the Oncology Department of Colțea Clinical Hospital, whilst a minority were registered and treated within the local oncology service. The main treatment options were combination therapies (cisplatin + 5-FU + cetuximab or carboplatin + 5-FU or paclitaxel + carboplatin or cisplatin + 5-FU), which were more common, as well as monotherapy with cisplatin, cetuximab or nivolumab. When cisplatin was administered, due to renal toxicity, the glomerular filtration rate and other indicators of renal function were constantly monitored. Treatment tolerance varied, but was generally poorer compared with radiotherapy. Among the most common and severe adverse reactions were: anaemia, neutropenia, hypomagnesaemia, thrombocytopenia, hyponatraemia, hypoalbuminaemia, lymphopenia and peripheral neuropathy.

Palliative chemotherapy was initiated for all 8 patients in the study who were diagnosed with tumour recurrence; it was also indicated in some cases where tumour progression was observed whilst the patients were receiving multimodal therapy.

The mortality rate for the entire cohort was approximately 67 per cent; the mean survival was 3 years and the median survival was 25 months. 13.3 per cent of patients diagnosed with HPV-positive oropharyngeal cancer experienced tumour recurrence. The proportion of patients treated for HPV-negative oropharyngeal cancer who experienced a recurrence was 12.5%. The 5-year survival rate for patients with HPV-positive oropharyngeal cancer was 60%, whilst for HPV-negative oropharyngeal cancer, the survival rate was only 27%. These findings are partially confirmed in the specialist literature: the risk of death decreases by 28%, and the risk of recurrence decreases by 49% in patients diagnosed with and treated for HPV-positive oropharyngeal cancers, compared with those with HPV-negative oropharyngeal cancers (Berman TA, 2017) .

During the case selection phase, we also identified patients diagnosed with laryngeal papillomatosis. Given the benign nature of the condition, these patients were not included in the study. However, recurrent laryngeal papillomatosis warrants attention due to its unpredictable course, the potential for spread to other segments of the upper respiratory tract or, in rare cases, malignant transformation into squamous cell carcinoma (POPESCU Dan, 2026) . Given the impact of such a condition on quality of life through its effect on respiratory and phonatory functions, the importance of vaccination is indisputable.

The first nationwide large-scale HPV vaccination programme took place in 2008, but was discontinued in 2011. The lack of public information and health education campaigns, the spread of anecdotal theories, fears regarding the safety of the vaccine, and the general reluctance of the population towards vaccination were the main reasons behind its failure.

In 2020, the Romanian Ministry of Health launched a new HPV vaccination campaign. With regard to the legal framework, GPs are responsible for HPV vaccination in Romania, including access to free HPV vaccines, which are supplied by the Ministry of Health upon their request (Manolescu, et al., 2022) .

HPV vaccination is currently included in the National Immunisation Programme, and from October 2025, free vaccination has been extended to girls and boys aged between 11 and 26. Furthermore, for women aged between 27 and 45, 50 per cent of the cost of the vaccine is reimbursed (HPV VACCINATION) (POPESCU Dan. T. M., 2026) .

Testing for HPV infection is not necessary prior to vaccination, because even in the event of a pre-existing HPV infection in the body, the vaccine provides protection against the other serotypes included in the vaccine, with no evidence that this protection diminishes over time (HPV VACCINATION) .

In our country, Gardasil-9 is the vaccine currently in use. It is a nonavalent vaccine, which offers protection against nine strains of HPV, namely: 6, 11, 16, 18, 31, 33, 45, 52 and 58, most of which are high-risk oncogenic strains.

In an analytical model incorporating TNM staging and surgical treatment, we observe differences between the simple and multiple models. In patients who did not undergo radical surgery, the effect becomes statistically significant when we take TNM stage into account; this influence can be attributed to the negative association between predictors – in patients with more advanced stages, radical surgery is not possible; furthermore, the HR increases for stages 3 and 4 in the multiple models, a trend which can also be explained by the negative association between the predictors.

The analysis model for all treatment modalities suggests that radiotherapy appears to be more beneficial when combined with surgery intended to be radical.

There were differences between the single- and multiple-model analyses for p16 status and N3 stage; our analysis reveals that patients who are p16-negative, at N3 stage and with Ki67 expression levels above 80% have the most severe disease progression. The correlation between biological markers and lymph node status warrants further research to identify associated prognostic factors.

Patients for whom radical surgery was possible had a median survival (RMST) at 12 months that was 2.3 months longer – a statistically significant difference – an RMST at 24 months that was 4.8 months longer, and an RMST at 5 years that was 10 months longer, although the latter difference was at the borderline of statistical significance.

In the 12-month analysis, the RMST was more than 3 months longer for radiotherapy; the 24-month analysis showed a mean survival time 7 months longer for radiotherapy; and at 60 months, the differences were not statistically significant.

7. Conclusions and personal contributions

7.1. Conclusions

This study was conducted over an average period of time (~3 years), which largely overlapped with the Covid-19 pandemic. The patient sample was relatively small, mainly due to the logistical challenges faced by the healthcare system and society between 2020 and 2022. Some patients did not complete their full course of treatment at Colțea Hospital or preferred to attend follow-up appointments at local ENT or oncology departments.

The research was driven by the global concern regarding the alarming rise in the incidence of HPV-positive oropharyngeal cancers, in particular.

Romania has one of the highest rates of oropharyngeal cancer amongst men in Europe, with approximately 11 new cases diagnosed per 100,000 men per year, whilst at the same time having the lowest vaccination rate in Europe, at under 10 per cent. Personally, I believe these figures call for a two-pronged action plan:

- taking steps to implement a national screening programme for oropharyngeal cancer,
- campaigns to promote HPV vaccination in schools, online, during news programmes, via billboards, and at public gatherings.

Last but not least, the spread of pseudoscientific theories should be halted by qualified medical professionals refuting them with sound arguments.

The favourable prognosis of HPV-positive oropharyngeal cancer compared with that of HPV-negative oropharyngeal cancer was confirmed in this study, supporting the data in the specialist literature; however, this is no reason to neglect the spread of this disease.

The AJCC Manual is the global medical standard used by surgeons and clinicians for the classification and staging of cancer, and in 2018, HPV-positive oropharyngeal cancer was recognised as a completely distinct condition—biologically, epidemiologically and in terms of prognosis—from HPV-negative oropharyngeal cancer.

In this study, restaging was carried out retrospectively due to a lack of information regarding real-time immunohistochemical diagnosis at the time of initiating therapies in conjunction with surgical treatment. This aspect may be considered a limitation of the study, but at the same time it may provide an impetus for future scientific work investigating how

immunohistochemical diagnosis influences the therapeutic strategy in HPV-positive oropharyngeal cancer.

7.2. Personal contributions

1. The most common grade of tumour differentiation encountered in cases of oropharyngeal cancer is well-differentiated squamous cell carcinoma, G1.
2. The most common site for cancer of the upper aerodigestive tract is the oropharynx, primarily affecting the tonsils, followed by the base of the tongue.
3. The course of cancer of the upper aerodigestive tract was more severe in males.
4. For early-stage cancer (stages I and II), the median 12-month survival was more than 2 months longer, the 24-month median survival was 8 months longer, and the 5-year survival was 30 months longer.
5. There is a positive association between the percentage of Ki67 expression and the risk of death; an increase of 1 is associated with a 2% increase in the risk of death.
6. There is evidence that in patients with Ki67 expression levels above 80%, the course of the disease is more severe, although the differences are at the borderline of statistical significance.
7. The mean survival was 3 years across the entire patient cohort, and the median survival was 25 months.
8. Radiotherapy appears to be more beneficial when combined with radical surgery.
9. With regard to p16 status and N3 stage, our analysis reveals that patients who are p16-negative, at N3 stage and with Ki67 expression levels above 80% have the most severe disease progression.
10. Patients for whom radical surgery was possible had a median restricted survival time (RMST) at 12 months that was 2.3 months longer, a statistically significant difference; the RMST at 24 months was 4.8 months longer and at 5 years 10 months longer, although this effect was at the borderline of statistical significance.
11. At 12 months, the median restricted survival time was more than 3 months longer for radiotherapy, and at 24 months, the RMST was more than 7 months longer.

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