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Accuracy of pre-, and posttreatment imaging of head and neck cancers, with special regard to HPV associated oropharyngeal cancers

PhD thesis

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List of Abbreviations

OPSCC - Oropharyngeal Squamous Cell Carcinoma

HPV - Human Papillomavirus

HNSCC - Head and Neck Squamous Cell Carcinoma

HNC - Head and Neck Cancer

SCC - Squamous Cell Carcinoma

DNA - Deoxyribonucleic Acid

TP53 - Tumor Protein p53 Gene

p53 - Tumor Protein p53

PD-L1 - Programmed Death-Ligand 1

PD-L2 - Programmed Death-Ligand 2

pRb - Retinoblastoma Protein

IHC - Immunohistochemistry

TILs - Tumor-Infiltrating Lymphocytes

TNM - Tumor, Node, Metastasis Classification

TNM8 - 8th Edition of the TNM Classification

TNM9 - 9th Edition of the TNM Classification

NCCN - National Comprehensive Cancer Network

NI-RADS - Neck Imaging Reporting and Data System

CT - Computed Tomography

MRI - Magnetic Resonance Imaging

FDG - Fluorodeoxyglucose

18F-FDG - 18F-Fluorodeoxyglucose

PET - Positron Emission Tomography

PET/CT - Positron Emission Tomography / Computed Tomography

ESMO - European Society for Medical Oncology

US - Ultrasound

FNAC - Fine-Needle Aspiration Cytology

MTB - Multidisciplinary Tumor Board

DOI - Depth of Invasion

ENE - Extranodal Extension

CRT - Chemoradiotherapy

SUV - Standardized Uptake Value

SUVmax - Maximum Standardized Uptake Value

SUVmean - Mean Standardized Uptake Value

VOI - Volume of Interest

MTV - Metabolic Tumor Volume

TLG - Total Lesion Glycolysis

PD-1 - Programmed Death-1

TPS - Tumor Proportion Score

CPS - Combined Positive Score

UICC - Union for International Cancer Control

SPSS - Statistical Package for the Social Sciences

cTNM - Clinical Tumor, Node, Metastasis Classification

pTNM - Pathological Tumor, Node, Metastasis Classification

PCR - Polymerase Chain Reaction

ctHPVDNA - Circulating Tumor HPV DNA

OSEM - Ordered Subset Expectation Maximization

FFPE - Formalin-Fixed, Paraffin-Embedded

MIP - Maximum Intensity Projection

ECOG - Eastern Cooperative Oncology Group Performance Status

PPV - Positive Predictive Value

NPV - Negative Predictive Value

OS - Overall Survival

DFS - Disease-Free Survival

DMFS - Distant Metastasis-Free Survival

CI - Confidence Interval

HR - Hazard Ratio

RR - Risk Ratio

OR - Odds Ratio

Gy - Gray (unit of absorbed radiation dose)

MBq - Megabecquerel (unit of radioactivity)

5-FU - 5-Fluorouracil

1. Introduction

1.1 Epidemiology

Head and neck cancers, -particularly those of the oral cavity, oropharynx, hypopharynx, and larynx- represent a major global health problem. Hungary ranks among the countries with the highest incidence and mortality rates of these malignancies worldwide, occupying the first place in incidence and the third place in mortality. These epidemiological data highlight the importance of improving our understanding of these diseases (1).

In recent years, the epidemiology of oropharyngeal squamous cell carcinoma (OPSCC) has undergone a profound shift due to the increasing prevalence of human papillomavirus (HPV) associated cases. HPV-positive OPSCC represents a distinct clinical and biological entity, characterized by younger age at onset, improved treatment response, and more favorable prognosis compared to HPV-negative OPSCC (2). However, despite its better outcomes, the rising incidence of HPV-related OPSCC contributes substantially to the growing global burden of head and neck cancers, particularly in countries like Hungary, where both traditional risk factors and viral oncogenesis coexist. Clarifying the prognostic and predictive factors underlying these tumor subtypes helps improve patient management and survival.

1.2 Main risk factors

The development of head and neck cancers is typically multifactorial. Tobacco use and alcohol consumption are well-established risk factors for HNSCC, acting both independently and synergistically in increasing cancer risk, with previous studies demonstrating that the risk of developing oral and pharyngeal cancer may increase more than 35-fold among individuals who smoke heavily and consume large amounts of alcohol (3, 4).

Tobacco smoke contains numerous carcinogenic compounds, such as polycyclic aromatic hydrocarbons and nitrosamines, which form DNA adducts and induce mutations in tumor suppressor genes - most notably in the *TP53* gene, encoding the p53 protein.

Alcohol enhances this process both by acting as a solvent that facilitates the penetration of carcinogens and through its metabolite, acetaldehyde, which directly damages DNA and interferes with repair mechanisms. The combined effects of these pathways contribute to genomic instability and malignant transformation of the mucosa.

Together, these classic risk factors account for nearly three-quarters of all cases (5). Among the remaining proportion, HPV infection plays a major etiologic role, with its contribution to oropharyngeal cancer varying considerably across countries. In Hungary, according to not-yet-published national data, HPV-related OPSCC represents a growing subset of head and neck cancers: 48.4% of OPSCC cases were HPV DNA-positive and 47.3% were p16-positive, showing a 93.5% concordance between the two markers.

Importantly, tobacco smoking remains an independent prognostic factor associated with both overall survival and progression-free survival in the entire HNSCC population, including patients with HPV-associated OPSCC. The risk increases by approximately 1% for each additional pack-year of tobacco exposure, regardless of HPV status (2).

High-risk HPV infection, predominantly HPV16, is the principal viral cause of OPSCC, and sexual behavior plays a major role in its transmission, with an increased risk associated with having a history of six or more lifetime sexual partners, four or more lifetime oral sex partners, and an earlier age at sexual debut (6, 7).

1.3 The role of HPV

The oropharynx - particularly the palatine and lingual tonsils - offers favorable conditions for viral persistence through its crypt architecture and reticulated epithelium, which facilitate access of the virus to the basal epithelial layer and create an immune-privileged microenvironment (8).

Persistent infection with oncogenic HPV types leads to expression of the viral oncoproteins E6 and E7. The E6 oncoprotein inactivates the tumor suppressor protein p53, while pRb inactivation by the HPV E7 oncoprotein results in dysregulated cell-cycle progression and inhibition of apoptosis. As compensatory feedback to pRb loss, overexpression of p16 occurs, which serves as a reliable surrogate marker of HPV-driven oncogenesis in IHC analyses. Because of its simplicity and affordability, IHC analysis has become widely used in clinical practice instead of HPV DNA typing (2, 9).

HPV-positive OPSCCs are characterized by a higher degree of tumor-infiltrating lymphocytes (TILs) and increased PD-L1 expression, indicating a distinct tumor immune microenvironment compared with HPV-negative cases (10).

HPV status of the tumor is a strong and independent predictor of survival in patients with OPSCC (2). However, it is important to note that this prognostic advantage is dependent

on the primary tumor site: it confers a clear survival benefit in OPSCC but not consistently in non-oropharyngeal cancers (10).

The more favorable prognosis observed in HPV-positive OPSCC compared with HPV-negative disease is multifactorial. Patients with HPV-positive tumors tend to be younger and have fewer comorbidities, and second primary tumors - which are largely associated with tobacco exposure - occur less frequently in this subgroup, consistent with their lower prevalence of smoking. In addition, HPV-positive cancers appear to harbor fewer genetic alterations and exhibit greater radiosensitivity, partly due to preservation of intact apoptotic pathways and a more active antitumor immune response, resulting in improved therapeutic outcomes. The higher survival rates are also attributed to better locoregional control, reflecting increased intrinsic sensitivity to radiation or enhanced radiosensitization with cisplatin (11). Currently, ongoing research is investigating whether treatment de-escalation could be safely implemented in HPV-positive OPSCC due to its generally favorable response to therapy. However, a major challenge remains the identification of the approximately 15% of patients who will develop recurrent disease and therefore require more intensive treatment rather than de-intensification (12).

Differences in OPSCC subtypes were recognized in the American Joint Committee on Cancer TNM8 staging guidelines, which recommended stratification by HPV status to improve staging, and it has further been refined in the 9th version of TNM classification (13).

Currently, no effective screening method exists for the early detection of HPV related OPSCC; therefore, large-scale vaccination programs remain the only approach to achieve widespread prevention (12). A 2021 systematic review provided evidence of an 82.7% relative reduction in oral and oropharyngeal HPV infections following HPV vaccination (14). Vaccination strategies vary across countries. In Hungary, since 2014, seventh-grade girls - and since 2020, 12-year-old boys as well - have been eligible to receive the HPV vaccine free of charge as part of the national immunization program, using the nonavalent formulation.

1.4 Possible prognostic factor: patient delay

Our clinical practice shows that head and neck cancer patients very often seek medical attention only with life-threatening symptoms. This primarily reflects the advanced cancer stage at presentation, which is well known to be associated with poorer survival outcomes.

Diagnostic delay refers to the total time from symptom onset to histological or radiological confirmation of diagnosis and can be divided into two distinct components: patient delay, representing the period between the onset of symptoms and the patient's first medical consultation, and system delay, referring to the time elapsed from first healthcare contact to definitive diagnosis or treatment initiation (Figure 1). In Hungary, governmental measures such as the 14-day rule (introduced in 2014 to reduce waiting times for staging imaging) and the 21-day rule (limiting referral time to multidisciplinary tumor boards) aim to minimize system-level delay. However, patient delay is far less influenced by healthcare organization and can be effectively reduced only through education and screening.

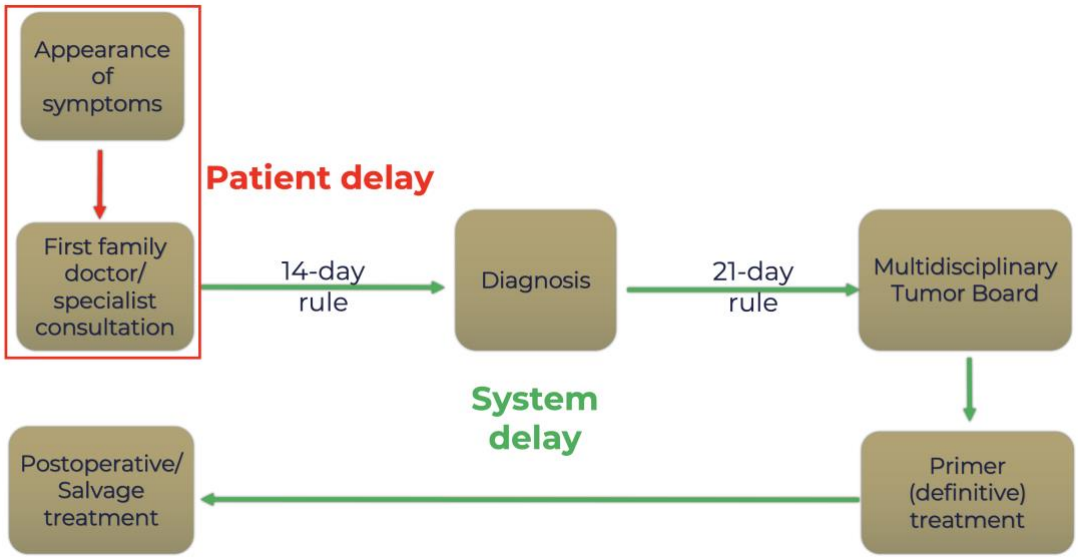


Figure 1. Schematic overview of patient and system delays along the head and neck cancer care pathway.

1.5 Prognostic role of the diagnostic work up

1.5.1 Staging

According to current NCCN (National Comprehensive Cancer Network) guidelines, initial staging should include contrast-enhanced CT and/or MRI of the primary tumor and neck, depending on the location of the primary tumor. For cervical lymph node assessment, both contrast-enhanced CT and MRI may be employed, with the selection of modality guided by the imaging requirements of the primary tumor. Nevertheless, it should be emphasized that, in the detection of nodal metastases, FDG-PET/CT provides the highest diagnostic accuracy compared with the other imaging modalities described. In line with ESMO guidelines, FDG-PET/CT as part of staging is recommended particularly in advanced-stage disease and in cases of carcinoma of unknown primary, while its routine use in early-stage disease is less emphasized compared with NCCN recommendations (15, 16).

In the presence of a neck mass, ultrasound-guided fine-needle aspiration cytology (US-guided FNAC) is the first-line diagnostic procedure for confirming the type of the lymph node malignancy. p16 immunohistochemistry (IHC) can be performed on cytological specimens when indicated. If FNAC is non-diagnostic or if additional tissue is required - particularly when systemic therapy, including immunotherapy, is being considered - core needle biopsy may be performed.

Distant metastatic disease should be evaluated using FDG-PET/CT or contrast-enhanced chest CT according to NCCN guidelines. In line with this, ESMO recommends at least a chest CT to exclude pulmonary metastases or a second primary lung tumor, especially in patients at high risk due to smoking. The combined use of contrast-enhanced chest CT and FDG-PET/CT provides higher diagnostic sensitivity than either modality alone (15, 16).

The main objective of imaging in the diagnostic work-up is to establish the stage of the disease, thereby ensuring the most appropriate therapeutic planning by the multidisciplinary tumor board (MTB).

Staging imaging provides detailed information on tumor size and local extent, including cartilage invasion and vocal cord mobility in laryngeal and hypopharyngeal carcinomas, depth of invasion (DOI) in oral cavity carcinomas, as well as invasion of critical surrounding structures such as the skull base, carotid artery, or prevertebral fascia,

corresponding to the T stage. It also allows assessment of nodal disease, including the presence, number, size, and laterality (unilateral or bilateral) of neck lymph node metastases, as well as lymph node extranodal extension (ENE) and involvement of surrounding anatomical structures, corresponding to the N stage. Extranodal extension (ENE) represents an independent adverse prognostic factor in HPV-negative tumors according to the 8th edition of the TNM classification (17).

Functional imaging techniques, primarily FDG-PET/CT, may further provide information on tumor metabolic activity and characteristics associated with tumor hypoxia. Parameters derived from these techniques - such as tumor volume and imaging features related to hypoxia - are increasingly recognized as important prognostic factors in assessing outcomes in patients with head and neck cancers.

1.5.2 Restaging

Post-treatment management of HNSCC remains a significant clinical challenge; imaging plays a crucial role in the early detection of residual or recurrent disease. NCCN guidelines recommend post-treatment imaging to assess treatment response and guide further management. Contrast-enhanced CT or MRI is commonly used for the evaluation of the primary tumor site and neck, while additional imaging modalities, including PET/CT may be applied for the assessment of cervical lymph nodes and potential distant metastases (15).

Follow-up imaging is generally recommended approximately 3-4 months after completion of definitive therapy in asymptomatic patients. Earlier imaging, typically within 4-8 weeks after treatment, may be considered when there is clinical suspicion of persistent disease or incomplete treatment response. The interpretation of imaging findings in the early post-treatment period requires careful consideration, as treatment-related inflammatory changes may complicate the differentiation between residual tumor and post-therapeutic effects; therefore, evaluation by radiologists experienced in head and neck imaging is highly beneficial (15).

1.6 The role of PET/CT

As mentioned above, FDG PET/CT is widely used for staging, treatment planning, and response monitoring in HNSCC; in addition, several studies have investigated its prognostic associations.

Primary staging

In primary staging, FDG PET/CT has demonstrated diagnostic superiority in clinically node-negative (cN0) HNSCC, with reported positive and negative predictive values of 62% and 83%, respectively (15).

Treatment planning

Recent prospective data demonstrate that FDG PET/CT significantly influences TNM staging and improves target volume delineation of both primary tumors and nodal regions in radiotherapy planning for HNSCC. Compared with contrast-enhanced CT alone, PET/CT provides more accurate target definition and may detect occult distant metastases (18).

Post-therapeutic evaluation and restaging

Following definitive radiotherapy or chemoradiotherapy, current guidelines recommend PET/CT for treatment response assessment and detection of residual or recurrent disease (15). PET/CT has a high negative predictive value for detecting residual disease, allowing reliable exclusion of persistent tumor and helping to avoid unnecessary diagnostic or therapeutic interventions (19). In addition, a meta-analysis of seven studies including 577 scans demonstrated high diagnostic accuracy of PET-based imaging for detecting residual or recurrent cancer, with pooled sensitivity and specificity of 86.2% and 82.3% at the primary site, 72.3% and 88.3% in the neck, and 84.6% and 94.9% for distant metastases, respectively. The optimal timing for post-treatment PET/CT is approximately 3-6 months after therapy, since imaging performed within the first 12 weeks can yield false-positive results due to post-radiation inflammation (20).

Cervical imaging after chemoradiotherapy (CRT) plays a crucial role in determining whether patients should undergo salvage neck dissection or can be managed with clinical observation. According to NCCN recommendations, if PET/CT reveals resectable, FDG-avid (or unequivocal) residual nodal disease, neck dissection is warranted (15). The randomized controlled PET-NECK trial demonstrated that a PET/CT-based surveillance strategy significantly reduced the number of neck dissections compared with planned neck dissection, without jeopardizing survival outcomes (21). Salvage neck dissection carries substantial morbidity - including wound complications, fistula formation, fibrosis, and long-term shoulder dysfunction - resulting in decreased quality of life; therefore, careful patient selection is essential (22).

PET/CT parameters

The standardized uptake value (SUV) is a semi-quantitative parameter used in FDG-PET imaging to estimate tissue glucose metabolism. It represents the activity concentration within a lesion normalized to the injected dose and body weight.

SUV max corresponds to the highest voxel-based SUV within a defined lesion and reflects the point of greatest metabolic intensity. In contrast, SUV mean represents the average SUV across all voxels within the defined volume of interest (VOI), thereby describing the mean metabolic activity within the lesion (23).

Intensity-based metabolic parameters, such as SUV max and SUV mean, describe the degree of tracer uptake within a given voxel or defined region, thereby reflecting

metabolic activity; however, they do not incorporate tumor volume and therefore do not represent overall tumor burden. In contrast, volumetric metabolic parameters, including metabolic tumor volume (MTV) and total lesion glycolysis (TLG), integrate both metabolic activity and tumor volume, allowing for a more comprehensive assessment of total tumor burden. MTV refers to the volume of FDG-avid lesions delineated using a threshold-based segmentation method. TLG is defined as the product of MTV and SUV mean.

PET/CT parameters as prognostic factors

Although SUV max is frequently - almost routinely - used in clinical PET assessment, its prognostic value in head and neck cancer remains controversial. Elevated pretreatment values, particularly in metastatic lymph nodes, have been associated with significantly worse disease-free, nodal progression-free, and distant metastasis-free survival, indicating that nodal SUV max may provide clinically meaningful information in selected settings (24). However, predictive strength appears to be context-dependent, and the strength of the association with overall survival (OS) is generally less consistent compared with volumetric metabolic parameters such as MTV and TLG. Consequently, volumetric parameters are widely regarded as more robust and reliable prognostic indicators (25). Meta analytic data support this observation, as pooled hazard ratios showed that MTV and TLG were associated with significantly worse event-free and overall survival compared to SUV max. Moreover, in univariate analyses, SUV max showed a correlation with overall survival in only 5 of 11 studies, whereas MTV was significant in 11 of 12 and TLG in 6 of 9 studies (26). Furthermore, independent studies have demonstrated that elevated MTV and TLG values are associated with poorer OS and disease-free survival (DFS). Specifically, higher MTV values were associated with approximately a four- to fivefold increase in the risk of disease recurrence and nearly a fourfold increase in overall mortality, while elevated TLG values were likewise linked to significantly increased risks of recurrence and death (27).

Other PET/CT-Based Prognostic Parameters

Beyond metabolic parameters, PET/CT may also provide additional prognostic information through certain morphologic features. One such parameter is the maximum tumor-to-node distance, which has been reported to correlate significantly with distant

metastasis-free survival (DMFS) and, when combined with clinical variables, may also help predict overall mortality (28).

Extranodal extension (ENE) is an adverse prognostic factor in HNSCC, associated with decreased survival and the need for intensified multimodal treatment. Therefore, accurate pretreatment detection is essential for optimal therapeutic planning. In this context, numerous studies have aimed to identify the most reliable imaging modality for ENE assessment, yet comparative analyses have produced heterogeneous findings. While pooled meta-analytic data suggest that MRI demonstrates the highest sensitivity for ENE detection and PET/CT provides high specificity owing to its metabolic component, some studies indicate that contrast-enhanced CT may achieve comparable or even superior specificity in selected clinical settings. From a clinical perspective, given the complementary strengths of anatomical and metabolic imaging, an integrated approach combining contrast-enhanced CT and/or MRI with FDG PET/CT appears to offer the most comprehensive evaluation of ENE in head and neck cancer (29, 30).

When evaluating the prognostic value of intratherapy and posttherapy PET/CT in head and neck cancer, positive findings were found to be strongly associated with adverse outcomes. Patients with positive scans had a more than threefold increased risk of death and nearly a fivefold increased risk of progression or recurrence. The relative risk of death within 2 years was particularly pronounced, exceeding sixfold, and although attenuated over longer follow-up, it remained statistically significant. Overall, posttherapy PET/CT demonstrated substantial prognostic value and appeared to provide stronger prediction of short-term mortality compared with intratherapy imaging (31).

1.7 PD-L1

Programmed death-ligand 1 (PD-L1) is an immune checkpoint ligand expressed on tumor cells and tumor-infiltrating immune cells that binds to the programmed death-1 (PD-1) receptor on T lymphocytes, thereby inhibiting T-cell-mediated antitumor immune responses. In HNSCC, PD-L1 expression represents a key mechanism of tumor immune evasion and serves as a clinically relevant predictive biomarker for response to immune checkpoint inhibitor therapy, such as pembrolizumab (32).

In clinical practice, PD-L1 expression guides the use of immune checkpoint inhibitors. Pembrolizumab is indicated as first-line treatment, either as monotherapy or in combination with platinum-based chemotherapy and 5-fluorouracil (5-FU), in recurrent or metastatic HNSCC with CPS ≥1. In addition, pembrolizumab monotherapy is approved for patients with TPS ≥50% whose disease has progressed on or after platinum-based chemotherapy (33).

2. Objectives

2.1 Patient delay and its clinical significance among head and neck cancer patients

Our primary objective was to define the mean and median patient delay of Hungarian head and neck cancer patients. Moreover:

H_0: Our hypothesis was that patient delay did not significantly influence the prognosis of HNSCC. Furthermore, we assumed that there were no significant differences between p16-positive and p16-negative patients in terms of the duration of patient delay, the initial symptoms, and clinical outcomes.

H_1: The alternative hypothesis assumed that the duration of patient delay influenced disease outcomes in HNSCC, moreover, differences could be observed between p16-positive and p16-negative patients in the length of patient delay, initial symptoms and clinical outcomes.

2.2 Accuracy of preoperative staging in the diagnosis of neck metastasis

H_0: We hypothesize that the diagnostic accuracy of the preoperative diagnostic work-up may be insufficient for detecting metastatic cervical lymph nodes.

H_1: The alternative hypothesis proposes that the diagnostic accuracy of preoperative investigations (CT, MRI, US, and FNAC) can be considered reliable for the detection of metastatic lymph nodes in patients undergoing elective neck dissection.

2.3 Accuracy of post-therapeutic PET/CT in terms of neck metastasis

As discussed in the previous chapter, the diagnostic performance of conventional imaging modalities in detecting lymph node metastases in HNSCC remains limited. Extending this line of investigation, the present study aimed to evaluate the performance of functional imaging techniques - specifically 18F-FDG PET/CT, regarded as the flagship of functional modalities - in the post-therapeutic setting.

H_0: The null hypothesis was the diagnostic performance of post-treatment PET/CT is limited in detecting residual cervical lymph node metastases. Moreover, the diagnostic accuracy of PET/CT does not differ significantly between HPV-positive and HPV-negative tumors.

H_1: The alternative hypothesis suggests that post-treatment PET/CT provides high diagnostic accuracy in detecting residual nodal metastases after definitive chemoradiotherapy (CRT), and that diagnostic performance differs between HPV-positive and HPV-negative tumors.

2.4. Correlation between PET/CT metabolic parameters, PD-L1 expression and p16/HPV status

FDG PET/CT is a widely accepted and highly reliable imaging modality, and several PET-derived metabolic parameters (such as SUVmax, MTV, and TLG) have been established as prognostic biomarkers. At the same time, immunotherapy has gained increasing importance in the management of head and neck cancers, particularly through PD-1/PD-L1 checkpoint inhibitors. Integrating these metabolic markers with immune and molecular characteristics may provide clinically meaningful insights in relation to p16/HPV status.

H_0: We hypothesized that PET/CT-derived metabolic parameters (SUVmax, MTV, TLG) were not associated with PD-L1 expression and p16/HPV status in oropharyngeal squamous cell carcinoma. Furthermore, we assumed that metabolic and immune characteristics did not differ between p16-positive, p16 and HPV DNA-positive and p16-negative tumors.

H_1: The alternative hypothesis states that PET/CT-derived metabolic parameters are significantly associated with PD-L1 expression or p16/HPV status in OPSCC. Furthermore, these parameters differ significantly among p16-positive, p16 and HPV DNA-positive, and p16-negative tumors.

3. Methods

3.1 Patient delay and its clinical significance among head and neck cancer patients

We included all patients diagnosed with squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx from September 2012 to August 2017 at the Department of Otorhinolaryngology, Head and Neck Surgery, Semmelweis University, Hungary.

The primary exclusion criteria included: patients not diagnosed via biopsy at our tertiary referral center, patients with primary tumors situated in other head and neck regions (nasopharynx, nasal cavity, paranasal sinuses, salivary glands, skin, or unknown primaries), and individuals with a history of previous treatment for head and neck malignancies or oncological therapy within the last five years. Furthermore, patients who had been diagnosed at other facilities were excluded.

This study involved a retrospective evaluation of patients' medical records, encompassing discharge summaries, multidisciplinary tumor board reports, histological reports, and imaging investigations.

Tumor staging was conducted in accordance with the 7th edition of the UICC TNM classification system (34).

HPV status was assessed by p16 immunohistochemistry (IHC). Detailed technical methodology is described in the corresponding Methods section.

The methodology of this study was based on the corresponding peer-reviewed publication (35).

3.2 Accuracy of preoperative staging examinations in the diagnosis of neck metastases

All patients participating in our study were histologically diagnosed and treated between September 2012 and August 2017 at the Department of Otorhinolaryngology, Head and Neck Surgery, Semmelweis University, Hungary. The patients received initial surgical intervention (resection of the primary tumor and neck dissection) for malignancies of the oral cavity, oropharynx, hypopharynx, or larynx.

Patients were excluded if they were not diagnosed at our tertiary referral center, had primary tumors located in other regions or of unknown origin, or had received prior head and neck oncological treatment. To ensure cohort homogeneity, patients undergoing salvage surgery or referred from other institutions were also excluded.

Patients received head and neck imaging (CT or MRI) to determine their cancer stage. All imaging investigations were conducted in accordance with Hungarian regulations at the regional imaging center designated by the patient's residence. Afterwards, patients were directed to the US-guided FNAC clinic of Department of Pathology, Forensic and Insurance Medicine, Semmelweis University to secure cytological confirmation of any suspicious lymph node identified by experienced cytologists. Both FNAC and imaging procedures were performed as part of routine clinical practice, involving different radiologists and cytologists.

Preoperative clinical staging (cTNM) was correlated with postoperative pathological staging (pTNM) based on histopathological findings of the neck dissection specimens, both determined according to the UICC 7th edition TNM Classification (2010) (34).

Upon examination of the radiological results, lymph nodes were identified as abnormal if they had already been recognized by the radiologist or were considered positive if they exceeded 10 mm in their smallest diameter, had central necrosis, or showed a round morphology.

The methodology of the preoperative diagnostic work-up study was based on the corresponding publication (36).

3.3. Accuracy of post-therapeutic PET/CT in terms of neck metastasis

3.3.1 Patients

The following prospective study was carried out also at the Department of Otorhinolaryngology, Head and Neck Surgery, Semmelweis University, from 2017 to 2025. Eligible patients had squamous cell carcinoma of the oropharynx, oral cavity, hypopharynx, or larynx with regional nodal metastases, confirmed with baseline staging PET/CT and underwent definitive chemoradiotherapy as their primary treatment.

The criteria for inclusion were completed concurrent chemoradiotherapy (CRT) containing at least one dose of cisplatin at 100 mg/m2 and radiotherapy with a total dose of 66-70 Gy, along with post-treatment 18F-FDG PET/CT imaging conducted at the Department of Nuclear Medicine at Semmelweis University.

All patients included underwent re-staging PET/CT following CRT. The positive nodal cases received salvage comprehensive neck dissection, followed by histological evaluation of the resected lymph nodes to confirm or exclude viable metastatic tumor. The pathological evaluation was conducted at Semmelweis University, Department of Pathology, Forensic, and Insurance Medicine.

Positive PET findings were correlated to histological results of the salvage neck dissection specimen. Conversely, nodal-negative cases lacked surgical confirmation and were consequently assessed through clinical and imaging follow-up for a minimum duration of two years. Patients exhibiting no signs of recurrence during this follow-up interval were deemed true negatives, while those who experienced regional recurrence were categorized as false negatives.

The exclusion criteria comprised: primary tumors situated outside the designated head and neck subsites, PET/CT examinations conducted at external institutions, non-PET post-treatment evaluations, absence of p16 staining for oropharyngeal cancer patients, or clinical follow-up of less than two years in PET-negative cases.

3.3.2 PET/CT acquisition protocol

Patients were given 2.5-3.0 MBq/kg of 18F-Fluorodeoxyglucose (18F-FDG) intravenously after a fasting duration of no less than 6 hours. Sixty minutes after injection, a low-dose, non-contrast CT scan and three-dimensional PET images were obtained from the jugulum to the mid-thigh. A subsequent focused scan of the head and neck region (extending from the frontal sinus to the aortic arch) was conducted with the arms positioned downward.

All scans were carried out using a hybrid PET/CT scanner (GE Discovery IQ5; GE Healthcare, Milwaukee, WI, USA). Image reconstruction was executed with CT attenuation correction, employing a traditional ordered subset expectation maximization (OSEM) technique with six iterations and six subsets, alongside a Bayesian penalized-likelihood reconstruction algorithm incorporating point-spread function modeling (Q.Clear, $\beta = 350$). For each scan, a standard PET image reconstruction was performed without CT attenuation correction. This method assists in eliminating misunderstanding of PET artifacts resulting from mistakes in CT attenuation correction, such as misregistration of CT and PET images due to patient movement and overestimation of FDG uptake caused by metallic implants in CT scans.

3.3.3 PET/CT image analysis

PET images were evaluated using two software platforms available at our institution, both providing fused PET/CT visualization and enabling semiquantitative analysis: GE AW Server™ (version 3.2 Ext. 3.4; GE Healthcare, Milwaukee, WI, USA) and Mediso InterView™ Fusion (version 3.10.9; Mediso Medical Imaging Systems, Budapest, Hungary). The visual analysis of tracer uptake on PET images was correlated with anatomical features on unenhanced CT scans.

PET/CT images were qualitatively analyzed, devoid of a systematic scoring methodology or established reference values for locoregional tracer uptake (e.g., jugular vein, liver, or mediastinal blood pool activity).

The patient's oncological medical history, occurrence of post-radiotherapy problems, and present symptoms were also considered for differential diagnosis. In reporting, it was essential to differentiate true positive findings from tracer accumulation of known non-oncologic origins (e.g., normal or elevated post-radiotherapy inflammation, ulceration,

osteo-/chondronecrosis, sialadenitis, and diffuse muscle uptake resulting from antalgic neck posture, among others).

Typically, moderate to severe uptake at the initial tumor site or nodal metastases, predominantly localized and/or asymmetric, is seen as a good indicator of residual disease. Indeterminate findings were also documented, necessitating either a follow-up scan after an additional three months or clinical rationale for continuing patient therapy.

3.3.4 p16 status

p16 IHC was used; technical details are provided in the relevant subsection.

The methodology of the post-treatment PET/CT study was based on the corresponding publication (37).

3.4 Correlation between PET/CT metabolic parameters, PD-L1 expression and p16/HPV status

3.4.1 Patients

The study population included patients diagnosed with oropharyngeal squamous cell carcinoma between 2017 and 2024 at the Department of Otorhinolaryngology, Head and Neck Surgery, Semmelweis University (38). Demographic, clinical, and pathological information was obtained from institutional electronic health records. All individuals underwent baseline staging with 18F-FDG PET/CT at the Department of Nuclear Medicine.

Tumor samples were processed at the Department of Pathology to determine PD-L1 expression and p16 status using immunohistochemical methods. Cases demonstrating p16 positivity were further subjected to HPV DNA analysis by real-time polymerase chain reaction (PCR) at the Department of Molecular Biology.

Exclusion criteria comprised PET/CT examinations performed outside our institution, lack of histopathological assessment at our tertiary referral center, and any history of prior oncologic treatment for head and neck cancer. Further reasons for exclusion included primary tumor diameter below 2 cm (owing to reduced reliability of PET-derived metabolic measurements related to partial volume effects), non-interpretable PD-L1 or p16 staining results, and histological types other than squamous cell carcinoma.

3.4.2 p16, HPV status determination

HPV status was primarily assessed by p16 IHC, as detailed in a later subsection. In addition, HPV DNA detection was performed in p16-positive cases using real-time polymerase chain reaction (PCR) to confirm the presence of viral DNA (39).

High-risk HPV genotyping was carried out following DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissue samples (QIAamp DNA FFPE Advanced Kit) and PCR amplification (GeneProof Human Papillomavirus Screening PCR Kit), enabling the detection of 23 high-risk HPV genotypes (HPV 16, 18, 26, 30, 31, 33, 34, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and 97). All procedures were performed according to the manufacturers' instructions.

3.4.3 PD-L1 Evaluation

PD-L1 expression was examined by IHC using the DAKO 22C3 pharmDx assay (Dako Denmark A/S, Glostrup, Denmark) on a Ventana Benchmark Ultra automated staining platform (Ventana Medical Systems, Inc., Tucson, AZ, USA). Formalin-fixed, paraffin-embedded tumor specimens were sectioned at 4 μm thickness and mounted on adhesive-coated glass slides (TOMO Adhesion Microscope Slide, Matsunami Glass Ind., Ltd., Kishiwada, Osaka, Japan). Tonsillar tissue served as a positive control, and case-specific negative control slides were also included.

Staining was carried out with the monoclonal mouse anti-PD-L1 antibody (clone 22C3), in accordance with the recommended protocol. Signal visualization was achieved using the OptiView DAB Detection Kit together with the OptiView Amplification Kit (Ventana Medical Systems, Inc., Tucson, AZ, USA), following a previously described protocol (40).

PD-L1 expression levels were quantified using both the Tumor Proportion Score (TPS) and the Combined Positive Score (CPS). TPS represented the percentage of viable tumor cells exhibiting partial or complete membranous staining at 200× magnification (20× objective), irrespective of staining intensity. CPS was calculated as the ratio of PD-L1-positive tumor cells and associated immune cells (lymphocytes and macrophages) to the total number of viable tumor cells, with a maximum value of 100.

Only viable tumor regions were considered for evaluation, while necrotic areas and immune cells associated with necrosis were excluded. A minimum threshold of 100 viable tumor cells was required to ensure evaluability. For tumor cells, positivity was defined exclusively by membranous staining, whereas in immune cells both membranous and cytoplasmic staining patterns were accepted.

All samples were independently assessed by two experienced pathologists (Gábor Lotz and Éva Kocsmár). In cases of discordant results, a consensus score was established following joint review.

3.4.4 PET/CT Imaging and Metabolic Parameter Analysis

Prior to imaging, patients fasted for at least 6 hours, after which 18F-FDG (2.5-3.0 MBq/kg) was administered intravenously. Image acquisition commenced approximately 60 minutes following tracer injection. A low-dose, non-contrast-enhanced CT scan was obtained first, followed by three-dimensional PET emission imaging covering the region from the jugular notch to the mid-thigh.

To achieve higher spatial resolution in the region of interest, an additional dedicated acquisition of the head and neck area was performed (extending from the frontal sinus to the aortic arch) with the patient's arms positioned alongside the body. In selected cases, imaging for radiotherapy planning was carried out using a flat tabletop in combination with a thermoplastic immobilization mask designed for head and neck fixation.

All examinations were performed on a hybrid PET/CT system (Discovery IQ5, GE Healthcare, Milwaukee, WI, USA). PET data were reconstructed using a Bayesian penalized likelihood algorithm incorporating point-spread function modeling (Q.Clear, $\beta = 350$). This reconstruction approach was applied uniformly across all scans and provides improved image quality while mitigating partial volume effects (41).

Image evaluation was conducted using dedicated fusion software (GE AW Server™ (version 3.2 Ext. 3.4; GE Healthcare, Milwaukee, WI, USA) and Mediso InterView™ Fusion (version 3.10.9; Mediso Medical Imaging Systems, Budapest, Hungary). Volumes of interest (VOIs) corresponding to the primary tumor and metastatic lymph nodes were defined using both semi-automated and manual segmentation techniques. Delineation was performed using a fixed absolute threshold (SUV >2.5) as well as a relative threshold set at 40% of SUVmax, in accordance with commonly applied methodologies reported in the literature (42). For quantitative evaluation, metabolic parameters were derived from manually refined VOIs using the fixed absolute threshold, as this approach showed the best agreement with anatomical tumor boundaries on CT (Figure 2). Areas of physiological or inflammatory FDG uptake were carefully excluded. Given the limitations of PET measurements in small lesions related to partial volume effects, cases with primary tumors <2 cm were not considered for metabolic analysis.

MTV was determined separately for the primary tumor, nodal metastases, and total tumor burden, while TLG was calculated based on the corresponding MTV and SUVmean values.

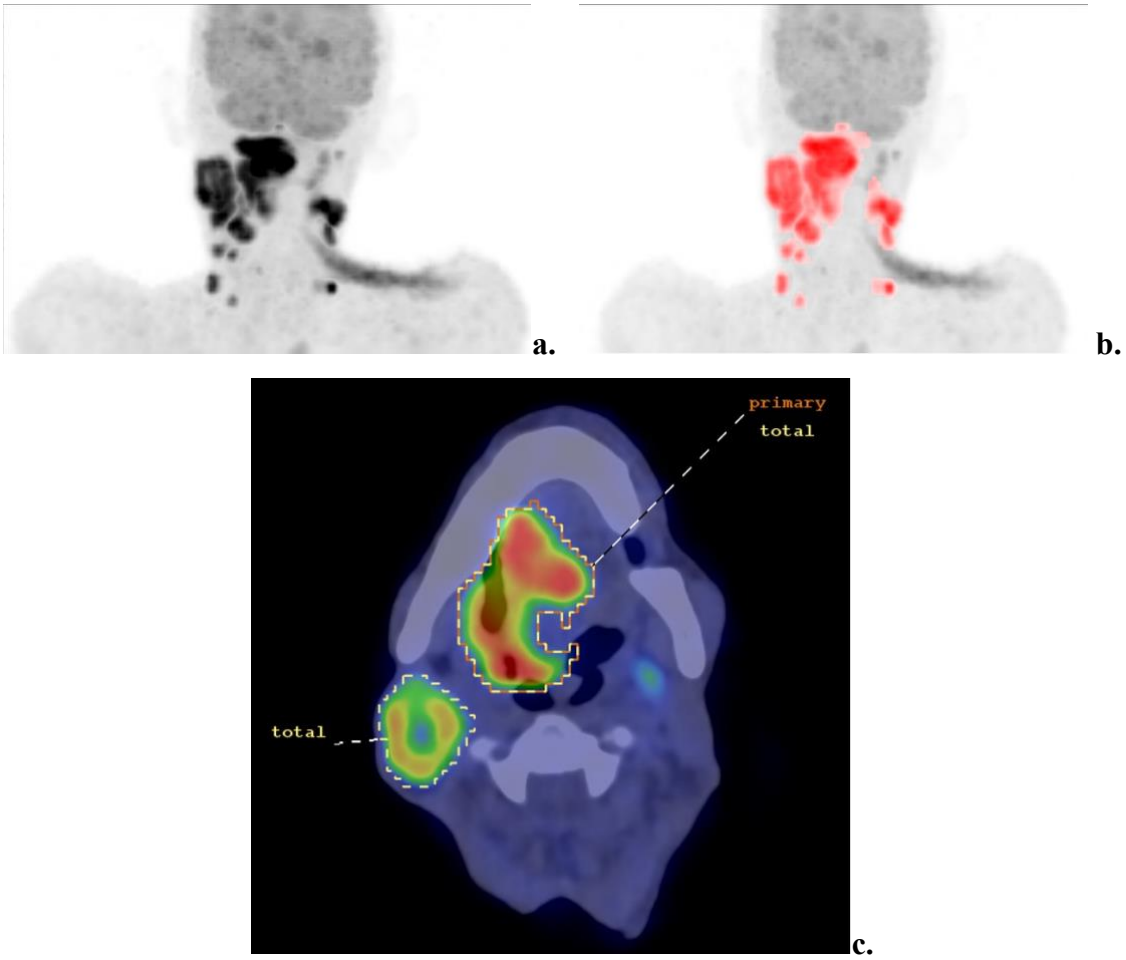


Figure 2. Tumor segmentation procedure. (38) a) original three-dimensional PET maximum intensity projection (MIP) image of the head and neck, intensive uptake of the meso-hypopharyngeal squamous cell carcinoma and the bilateral neck lymph node metastases; b) automatic segmentation of the total tumor volume with SUV low threshold of 2.5 g/ml (volumes of interest in red), this method was kept as reference, since it could not exclude every region with physiologic or inflammatory uptake close to the tumor; c) semi-automatic segmentation of the primary tumor (orange) and total tumor volume (yellow, primary and metastatic nodes together) using a SUV low threshold of 2.5 g/ml and manual correction if needed according to the anatomical landmarks on CT

3.5 Assessment of p16 Status

According to established clinical practice and guidelines, p16 immunohistochemistry (IHC) is recognized as a valid surrogate marker for HPV identification in oropharyngeal cancer (43).

The HPV status was evaluated by p16 IHC in all cases using the tumor suppressor $p16^{INK4}$ (Vitro Master Diagnostics; Spain, Sevilla, mouse anti-human p16-INK4A monoclonal antibody [MX007], diluted 1:100). The primary antibody was performed at 37°C for a duration of 10 minutes. A case was defined as p16-positive when a minimum of 70% of tumor cells exhibited powerful combined nuclear and cytoplasmic staining.

3.6 Statistical analysis

Statistical analyses were performed using a unified methodological approach. The data distribution was examined using normality tests (Kolmogorov-Smirnov and Shapiro-Wilk) and descriptive statistics (mean, median, and standard deviation). Non-parametric techniques were mostly employed because the majority of variables did not follow a normal distribution.

The Mann-Whitney U and Kruskal-Wallis tests were used for group comparisons, and the Chi-square or Fisher's exact test, depending on the situation, were used for categorical variable analysis. (Exceptions included age at diagnosis, staging SUVmax of tumor plus node and tumor alone, in the PD-L1 - PET/CT study (Section 4.4)).

Correlations between variables were assessed using Spearman’s rank correlation.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and subgroup analyses based on p16 status were used to assess diagnostic performance.

The Kaplan-Meier method was used to examine survival outcomes, and log-rank tests were used to compare the results. Cox regression analysis, both univariate and multivariate, were used to evaluate prognostic factors. Treatment response predictors were found using logistic regression models.

Statistical significance was defined as a p-value of less than 0.05. TIBCO Statistica 14.0 and SPSS (IBM Corp., Armonk, NY) were employed to perform statistical analyses.

3.7 Ethical approval

The Semmelweis University Institutional Ethics Committee approved all four studies (SE IKEB 105/2014).

3.8 Tumor staging

Tumor staging was conducted in accordance with the 7th edition of the UICC TNM classification system (34).

Table 1. Methodological characteristics of the four studies included in the dissertation, based on the related publications (35-38)

Study / Feature	Patient delay	Preoperative workup	Post-treatment PET/CT	PET metabolic parameters / PD-L1
Design	Retrospective	Retrospective	Prospective	Retrospective
Study period	2012-2017	2012-2017	2017-2025	2017-2024
Main institution	Department of Otorhinolaryngology, Head and Neck Surgery, Semmelweis University			
Collaborating units	Pathology, Forensic and Insurance Medicine	Pathology, Forensic and Insurance Medicine	Nuclear Medicine; Pathology, Forensic and Insurance Medicine	Nuclear Medicine; Pathology, Forensic and Insurance Medicine; Molecular Biology
Sample size	236	62	58	60
Study population	Oral cavity, oropharynx, hypopharynx, larynx; SCC	Oral cavity, oropharynx, hypopharynx, larynx; SCC; primary surgery + neck dissection	Oropharynx, oral cavity, hypopharynx, larynx; node-positive SCC; definitive CRT	Oropharynx; newly diagnosed OPSCC; baseline FDG PET/CT
Main objective	Clinical significance of patient delay	Accuracy of conventional imaging and FNAC for nodal metastases	Diagnostic performance of post-treatment FDG-PET/CT by p16 status	Association of PET metabolic parameters with PD-L1, p16/HPV, and outcome

Study / Feature	Patient delay	Preoperative workup	Post-treatment PET/CT	PET metabolic parameters / PD-L1
Inclusion criteria	Histologically confirmed SCC diagnosed at Semmelweis University	Histologically confirmed SCC treated with upfront surgery and neck dissection	Regional nodal metastasis on staging PET/CT; completed definitive CRT; institutional post-treatment PET/CT	Newly diagnosed OPSCC with baseline PET/CT and available tissue for PD-L1 and p16 assessment
Exclusion criteria	Outside diagnosis; other sites; unknown primary; previous HNC treatment; oncologic treatment within 5 years	Outside diagnosis; other sites; unknown primary; previous HNC treatment; salvage/referral cases	Outside PET/CT; non-PET post-treatment evaluation; missing p16 in OPSCC; < 2-year follow-up in PET-negative cases	Outside PET/CT or histopathology; previous HNC treatment; primary tumor < 2 cm; non-evaluable PD-L1/p16; non-SCC
HPV / p16 assessment	p16 IHC in OPSCC	No	p16 IHC in OPSCC	p16 IHC; HPV DNA PCR in p16-positive cases
PD-L1 assessment	No	No	No	PD-L1 IHC (TPS, CPS)

Study / Feature	Patient delay	Preoperative workup	Post-treatment PET/CT	PET metabolic parameters / PD-L1
Statistics	Kruskal-Wallis; Mann-Whitney U; Chi-square/Fisher; Kaplan-Meier; log-rank	Descriptive statistics; Chi-square/Fisher	Descriptive statistics; Kaplan-Meier; log-rank	Spearman; uni-/multivariate Cox; Kaplan-Meier; logistic regression
Ethical approval	SE IKEB 105/2014			

4. Results

4.1 Patient delay and its clinical significance among head and neck cancer patients

4.1.1 Findings in the overall study population

The study included 236 patients, representing the Hungarian head and neck cancer population. Among them, 198 were men and 38 were women, with a mean age of 61 years (range: 40-92 years) at diagnosis.

Patient delay showed considerable variability, with a median of 9.5 weeks (range: 0-209 weeks) and a mean of 17.57 weeks (standard deviation: 23.67). When analyzed according to tumor localization (Table 2), patient delay differed significantly across subsites (p=0.004). The longest delays were observed in laryngeal cancers, most notably in supraglottic tumors (median: 17.5 weeks), followed by glottic (15 weeks) and transglottic lesions (13 weeks). In contrast, patients with oral cavity and oropharyngeal cancers presented earlier, with a median delay of 8 weeks.

Table 2: Delay in weeks according to the region (p=0.004) (35)

Localization	Number	Mean patient delay (weeks)	Median patient delay (weeks)
Oral cavity	14	17.09	8
Oropharynx	75	11.18	8
Hypopharynx	51	13.9	11
Supraglottic larynx	24	27.75	17.5
Glottic larynx	60	25.9	15
Transglottic larynx	10	16.33	13
Subglottic larynx	1	Value= 4	

Presenting symptoms showed distinct localization-specific patterns (Table 3). Sore throat, hoarseness, neck mass, difficulty in swallowing, dyspnea, and earache showed localization-specific variation, whereas weight loss, hemoptysis, and trismus did not differ substantially across anatomical subsites. In oral cavity cancers, difficulty in swallowing was the leading complaint, while oropharyngeal tumors were mainly associated with sore throat, difficulty in swallowing, and neck mass. Hypopharyngeal cancers were characterized by difficulty in swallowing together with sore throat, hoarseness, neck mass, and earache. Supraglottic tumors showed a more heterogeneous symptom profile, whereas hoarseness predominated in glottic and transglottic laryngeal cancers.

Table 3: symptoms (in percentage) with significance value and median patient delay (in weeks) according to the region (35)

regions / complaints	oral cavity	oropharynx	hypopharynx	supraglottic larynx	glottic larynx	transglottic larynx	subglottic larynx	p
sore throat	28.6	**48.0**	25.5	29.2	3.3	0	0	<0.001
hoarseness	0	4.0	21.6	29.2	**96.7**	60.0	**100**	<0.001
neck mass	14.3	**32.0**	27.5	20.8	0	10.0	0	<0.001
difficulty in swallowing	42.9	40.0	**47.1**	25.0	0	20.0	0	<0.001
weight loss	**14.3**	4.0	9.8	4.2	0	0	0	0.149
earache	7.1	13.3	**19.6**	4.2	0	10.0	0	0.027
hemoptysis	0	0	2.0	4.2	0	0	0	0.513
trismus	0	2.7	0	0	0	0	0	0.636
dyspnea	0	5.3	5.9	4.2	3.3	20.0	100	<0.001
median patient delay in weeks	8.0	8.0	11.0	17.5	15.0	13.0	4.0	

Stage distribution at diagnosis also varied by tumor location ($p<0.05$), as shown in Table 4. Glottic cancers were predominantly detected at an early stage (67%), whereas tumors arising from other anatomical sites were more frequently diagnosed in a locoregionally advanced stage. In addition, distant metastases at presentation were observed in 10% of patients with oropharyngeal and hypopharyngeal cancers.

Table 4: cancer stage according to the cancer site: **p<0.05 (35)**

	Oral cavity	Oropharynx	Hypopharynx	Supraglottic larynx	Glottic larynx	Transglottic larynx
Early stage (T1-2N0M0)	3 23%	7 10%	5 10%	8 36%	**38** **67%**	3 30%
Locoregionally advanced stage (T3-4N0M0, T1-4N1-3M0)	10 77%	**55** **81%**	**40** **80%**	13 59%	18 32%	7 70%
Distant metastasis (T1-4N1-3M1)	0 0%	**6** **10%**	**5** **10%**	1 5%	1 2%	0 0%

Tumor localization was significantly associated with survival (log-rank p<0.001; Figure 3), with laryngeal cancers showing more favorable outcomes despite longer patient delay, whereas oral cavity and pharyngeal cancers were associated with poorer survival and shorter patient delay. In contrast, patient delay showed no significant association with survival when evaluated across delay quartiles (p=0.214; Figure 4). This was further supported by multivariate Cox regression analysis, in which stage was identified as a significant predictor of survival (HR=1.746, p<0.001), whereas patient delay was not (HR=0.998, p=0.553).

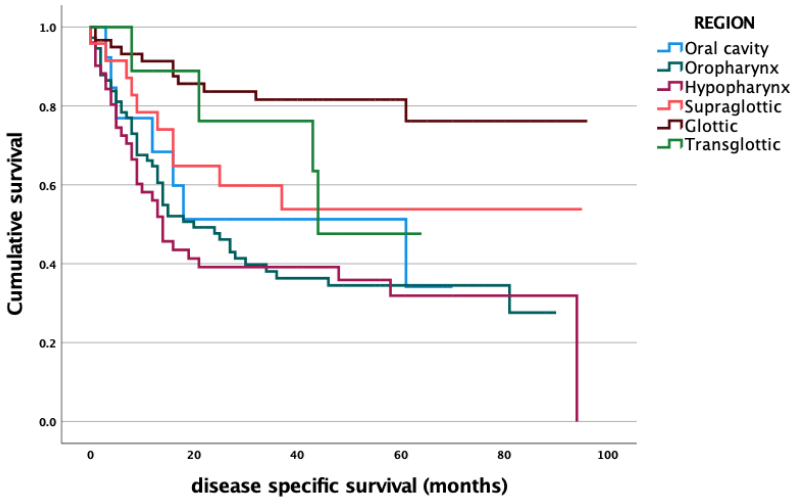


Figure 3: Primary tumor site and survival analysis log rank p < 0.001 (35)

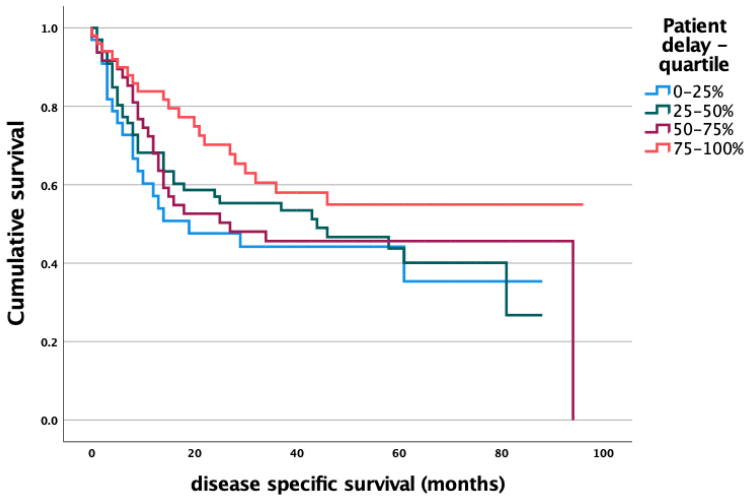


Figure 4: Patient delay and survival analysis log rank p = 0.214 (35)

4.1.2 Results according to p16 status in oropharyngeal cancer

Patient delay did not differ significantly according to p16 status in oropharyngeal cancer (p=0.524). Although the mean delay was longer in p16-positive patients (14.47 vs. 9 weeks), the median delay was identical in both groups (8 weeks) (Table 5).

A neck mass as presenting symptom was significantly more common in p16-positive patients compared to p16-negative cases (p=0.003). (Table 6). There was a tendency toward more frequent sore throat and swallowing difficulties in p16-negative cases, although these differences did not reach statistical significance.

The well-established survival difference according to HPV status was also observed in our cohort, with p16-negative OPSCC showing significantly worse survival (p = 0.032, Figure 5).

Table 5: p16-positivity and patient delay in weeks in oropharyngeal cancers p= 0.524 (35)

p16 status	Numbers (available patients delay scores)	Mean delay	Median delay
Positive	18 (17)	14.47 (2-56) weeks	8.00 weeks
Negative	28 (26)	9.81 (2-42) weeks	8.00 weeks
Not available	30		

Table 6: Oropharyngeal cancer symptoms according to p16 status (35)

	Presence of symptom in percent		p
	p16-positive OPSCC	p16-negative OPSCC	
Sore throat	38.9	64.3	0.091
Hoarseness	5.6	3.6	1.0
Neck mass	61.1	17.9	0.003
Difficulty in swallowing	16.7	42.9	0.064
Weight loss	0	7.1	0.513
Earache	0	17.9	0.140
Hemoptysis	-	-	-
Trismus	5.6	3.6	1.0
Dyspnea	0	14.3	0.144
Median patient delay in weeks	8 weeks	8 weeks	0.524

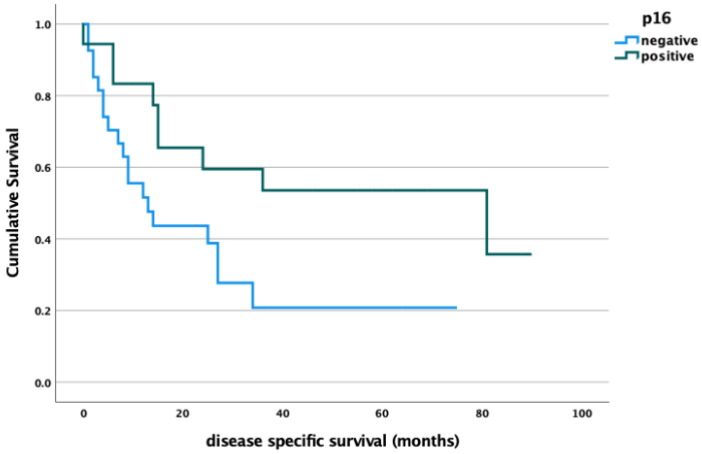


Figure 5: p16 status and survival analysis log rank (p = 0.032) (35)

Taken together, these results support retaining the null hypothesis in terms of correlations between prognosis and patient delay, whereas rejecting the null hypothesis regarding differences in the presenting symptoms of p16-positive and negative oropharyngeal cancers.

4.2. Accuracy of preoperative staging in the diagnosis of neck metastasis

Among the 62 patients examined, 54 were men and 8 were women, with a mean age of 61 years (range: 43-77 years). Primary tumor distribution is shown in Table 7, with hypopharyngeal and supraglottic cancers being the most common.

Table 7: Distribution of tumors by localization (36)

localization	%
oral	3.2
oropharynx	9.7
hypopharynx	32.3
supraglottic	22.6
glottic	9.7
transglottic	21
subglottic	1.6

Tumor stage at presentation is summarized below**.** T1 tumors were rare (4.8%). Most cases were T2 or T3 (32.3% each), while T4a and T4b tumors accounted for 30.6%, indicating a predominance of advanced primary disease in this cohort.

For nodal status, 53.2% of patients were classified as N0. The rest showed nodal involvement, most often N1 and N2a (both 16.1%), followed by N2b (8.1%), N2c (4.8%), and N3 (1.6%). Accordingly, elective neck dissection was performed in 53.2% of cases, while 46.7% underwent therapeutic, comprehensive neck dissection.

Surgical procedures are summarized in Table 8, with partial or total laryngectomy combined with ipsilateral neck dissection being the most common approaches.

Table 8: Types of surgeries performed (36)

therapy	**%**
partial laryngectomy with ipsilateral neck dissection	27.4
total laryngectomy with ipsilateral neck dissection	38.7
pharyngotomy with ipsilateral neck dissection	8.1
transoral resection with ipsilateral neck dissection	6.5
partial laryngectomy bilateral neck dissection	4.8
total laryngectomy bilateral neck dissection	14.5

Regarding preoperative imaging, CT was used in 84% of cases, with MRI and ultrasound alone used in 10% and 3.2%, respectively.

Bilateral neck dissections were done in 12 instances where the preoperative FNAC values were identical for both sides of the neck. In two instances, contradictory FNAC results were obtained, with one side positive and the other negative, despite bilateral metastases being identified in the pathology report of the neck dissection specimen. Both instances were classified as false negatives in our research.

Radiological nodal staging showed a significant association with histopathological findings ($p<0.001$), where pathological lymph node status was categorized as pN-negative (pN0) and pN-positive (pN1-pN3). Despite this, discrepancies were observed: among patients classified as node-negative on imaging, 26.1% were found to be pN-positive on final pathology. In contrast, 17.1% of radiologically positive cases were pN-negative histologically (Table 9).

Table 9: Relationship between imaging report and pathological lymph node status (36)

%	pathology lymph node status (pN)	
lymph node status described on the imaging techniques / radiology	negative	positive
negative	73.9	26.1
positive	17.1	82.9

FNAC findings were significantly associated with histopathological lymph node status ($p<0.001$). However, a considerable proportion of false-negative results was observed: among cases classified as negative by FNAC, only 66.7% were confirmed as negative on histopathological examination of the neck dissection specimen. In contrast, all cytologically positive cases were verified histologically (Table 10).

Table 10: Relationship between FNAC results and pathological lymph node status (36)

%	pathology lymph node status (pN)	
FNAC	negative	positive
negative	66.7	33.3
positive	0	100

Taken together, the diagnostic performance parameters (sensitivity, specificity, positive and negative predictive values; Table 11) indicate that imaging modalities alone showed limited reliability, whereas FNAC proved to be highly reliable in confirming nodal metastasis when positive. However, the relatively high rate of false-negative results suggests that neither method is sufficient on its own.

Table 11: Percentage values for imaging (CT, MRI, US) and FNAC examinations (36)

%	imaging techniques	FNAC
sensitivity	82.8	81.8
specificity	73.9	100
positive predictive value	82.8	100
negative predictive value	73.9	66.6

The clinical TNM stage, including nodal status (cN), is established by the multidisciplinary tumor board based on the combined assessment of physical examination, imaging findings, and FNAC results.

In our cohort, a high concordance was observed for clinically node-positive cases, with 96.6% confirmed as pN-positive on histopathological examination. In contrast, among patients classified as cN0 preoperatively, only 69.7% were truly node-negative, corresponding to a false-negative rate of 30.3% (Table 12).

Table 12: Relationship between clinical and pathological lymph node status (36)

%	Pathology lymph node status (pN)	
Clinical nodal status (cN)	negative	positive
negative	69.7	30.3
positive	3.4	96.6

Taken together, these results support the null hypothesis, as the observed false-negative rates indicate that preoperative investigations cannot reliably exclude metastatic cervical lymph nodes.

4.3. Diagnostic performance of post-treatment PET/CT by HPV status

Of the 58 patients included in the analysis, 38 were men and 20 were women; the mean age was 60 years (range: 43-83 years). Most primary tumors were in the oropharynx (n=46). Fewer cases originated from the hypopharynx (n=5), oral cavity (n=4), and supraglottic larynx (n=3). Baseline clinical characteristics are shown in Table 13.

Patients were divided into two equal groups according to tumor biology. The first consisted of p16-positive oropharyngeal carcinomas (n=29, Group 1), while the second included p16-negative oropharyngeal tumors as well as cancers of the oral cavity, hypopharynx, and supraglottic larynx (n=29, Group 2). Tumor stage was defined based on the 7th edition of the TNM classification system (34).

Table 13: Descriptive statistics (37)

	Total	p16-positive Oropharynx (Group 1)	All, Except p16-positive Oropharynx (Group 2)
Tumor origin			
	58 (100%)	29 (50%)	29 (49%)
oral cavity			4 (7%)
p16 neg. oropharynx			17 (29%)
hypopharynx			5 (9%)
supraglottic larynx			3 (5%)
Age at diagnosis			
$x < 40$	0 (0%)	0 (0%)	0 (0%)
$40 \leq x < 50$	8 (14%)	7 (24%)	1 (3%)
$50 \leq x < 60$	17 (29%)	8 (28%)	9 (31%)
$60 \leq x < 70$	29 (50%)	11 (38%)	18 (62%)
$70 \leq x < 80$	3 (5%)	2 (7%)	1 (3%)
$80 \leq x < 90$	1 (2%)	1 (3%)	0 (0%)
$90 \leq x < 100$	0 (0%)	0 (0%)	0 (0%)
Missing	0 (0%)	0 (0%)	0 (0%)
Mean (in years) ± SD	60.22 ± 8.83	58.59 ± 10.71	61.85 ± 6.22
Sex			
Male	38 (66%)	18 (62%)	20 (69%)
Female	20 (34%)	11 (38%)	9 (31%)
Unknown	0 (0%)	0 (0%)	0 (0%)

Tobacco use			
Current	25 (43%)	8 (28%)	17 (59%)
Former	7 (12%)	3 (10%)	4 (14%)
Never	18 (31%)	12 (41%)	6 (21%)
Unknown	8 (14%)	6 (21%)	2 (7%)
Alcohol consumption			
Current	16 (28%)	5 (17%)	11 (38%)
Former	2 (3%)	1 (3%)	1 (3%)
Never	30 (52%)	17 (59%)	13 (45%)
Unknown	10 (17%)	6 (21%)	4 (14%)
ECOG			
0	43 (74%)	25 (86%)	18 (62%)
1	12 (21%)	4 (14%)	8 (28%)
2	2 (3%)	0 (0%)	2 (7%)
Unknown	1 (2%)	0 (0%)	1 (3%)
Primary tumor stage before irradiation			
1	7 (12%)	5 (17%)	2 (7%)
2	22 (38%)	16 (55%)	6 (21%)
3	11 (19%)	5 (17%)	6 (21%)
4a	12 (21%)	2 (7%)	10 (34%)
4b	6 (10%)	1 (3%)	5 (17%)
Unknown	0 (0%)	0 (0%)	0 (0%)
Node stage before irradiation			
0	not applicable		
1	27 (47%)	19 (66%)	8 (28%)
2a	1 (2%)	0 (0%)	1 (3%)
2b	9 (16%)	3 (10%)	6 (21%)
2c	8 (14%)	3 (10%)	5 (17%)
3	13 (22%)	4 (14%)	9 (31%)
Unknown	0 (0%)	0 (0%)	0 (0%)
TNM stage before irradiation			
1	16 (28%)	16 (55%)	0 (0%)
2	6 (10%)	6 (21%)	0 (0%)
3	10 (17%)	7 (24%)	3 (10%)
4a	14 (24%)	0 (0%)	14 (48%)
4b	12 (21%)	0 (0%)	12 (41%)
Unknown	0 (0%)	0 (0%)	0 (0%)
5-year survival			
yes	11 (19%)	6 (21%)	5 (17%)
no	2 (3%)	0 (0%)	2 (7%)
shorter surveillance	45 (78%)	23 (79%)	22 (76%)

PET/CT nodal tumor stage following irradiation			
positive	23 (40%)	7 (24%)	16 (55%)
negative	35 (60%)	22 (76%)	13 (45%)
Unknown	0 (0%)	0 (0%)	0 (0%)
follow up PET/CT result			
true positive	16 (28%)	3 (10%)	13 (43%)
true negative	34 (59%)	22 (76%)	12 (43%)
false positive	7 (12%)	4 (14%)	3 (10%)
false negative	1 (2%)	0 (0%)	1 (3%)
Unknown	0 (0%)	0 (0%)	0 (0%)

Post-treatment FDG-PET/CT had, across the entire study population, high sensitivity and NPV (94% and 97%), whereas specificity and PPV were lower in comparison (83% and 70%). When these results are examined in more detail according to p16 status, differences between the two groups become apparent. In the p16-negative subgroup, sensitivity, specificity, PPV, and NPV showed a relatively balanced distribution (93%, 80%, 81%, and 92%, respectively), without marked divergence between the individual parameters. In the p16-positive subgroup, sensitivity and NPV remained high (100% and 100%), while PPV was substantially lower (43%), with a limited ability to clearly separate post-treatment changes from residual malignancy. Specificity (85%) was comparable to that observed in the p16-negative group (Table 14).

Table 14: Percentage values for PET/CT (37)

%	total	p16-negative	p16-positive
sensitivity	94	93	100
specificity	83	80	85
positive predictive value	70	81	43
negative predictive value	97	92	100

A significant difference in survival was observed between PET/CT-positive patients, who underwent neck dissection due to residual disease, and PET/CT-negative patients, who did not require salvage surgery, with poorer outcomes in the PET/CT-positive group (log-rank p = 0.008) (Figure 6).

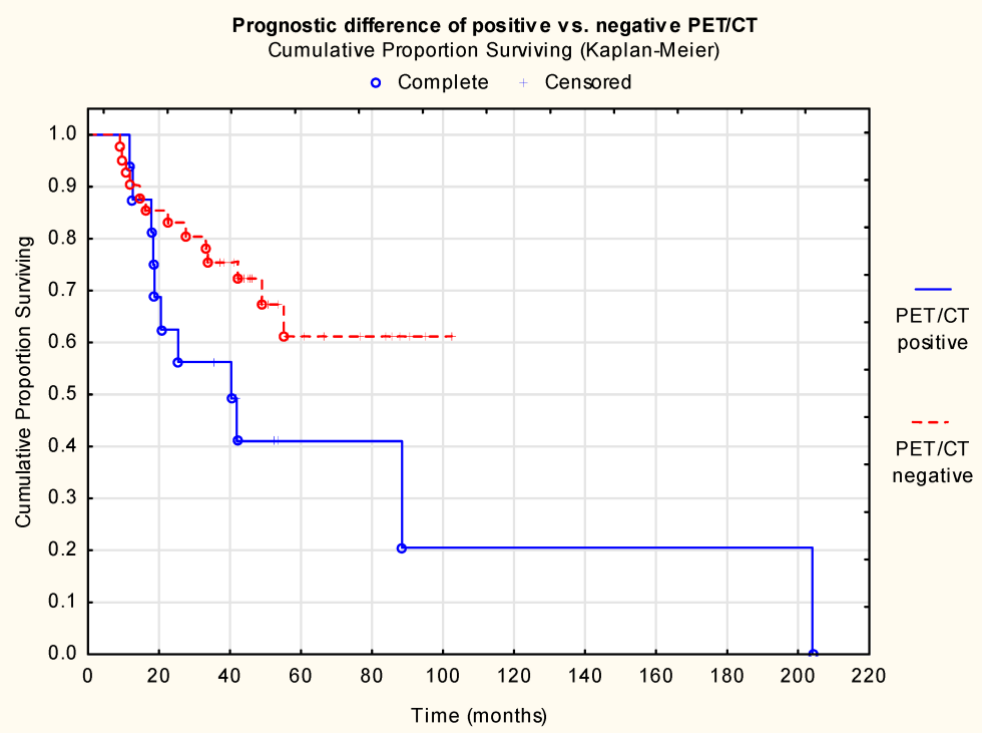


Figure 6: Prognostic difference in positive (confirmed by neck dissection) vs. negative (no recurrence in follow-up) PET/CT (37)

Cumulative proportion surviving (Kaplan-Meier) of patients with/without neck dissection (Log-Rank Test: WW = 5,963, Sum = 24.110, Var = 5.083, Test statistic = 2.645, p = 0.008), PET/CT positive (underwent neck dissection) n=17,

PET/CT negative (no neck dissection) n = 41, patients at risk n=58.

Survival analysis demonstrated a significant difference between patients with PET/CT-negative findings and no recurrence during follow-up (true-negative) and those with PET/CT-positive cases confirmed by nodal disease at neck dissection (true-positive) ($p < 0.001$) (Figure 7).

In contrast, no significant difference was observed between true-negative patients and those with PET/CT-positive findings without confirmed disease during follow-up (false-positive) ($p = 0.763$) (Figure 8).

The interval between completion of CRT and follow-up PET/CT averaged 15.7 weeks (median 13.5 weeks; range 10-33 weeks).

Postoperative complications were rare, with isolated cases of wound healing disorder and fistula formation (n = 1 each), as well as permanent marginal mandibular nerve palsy in two patients.

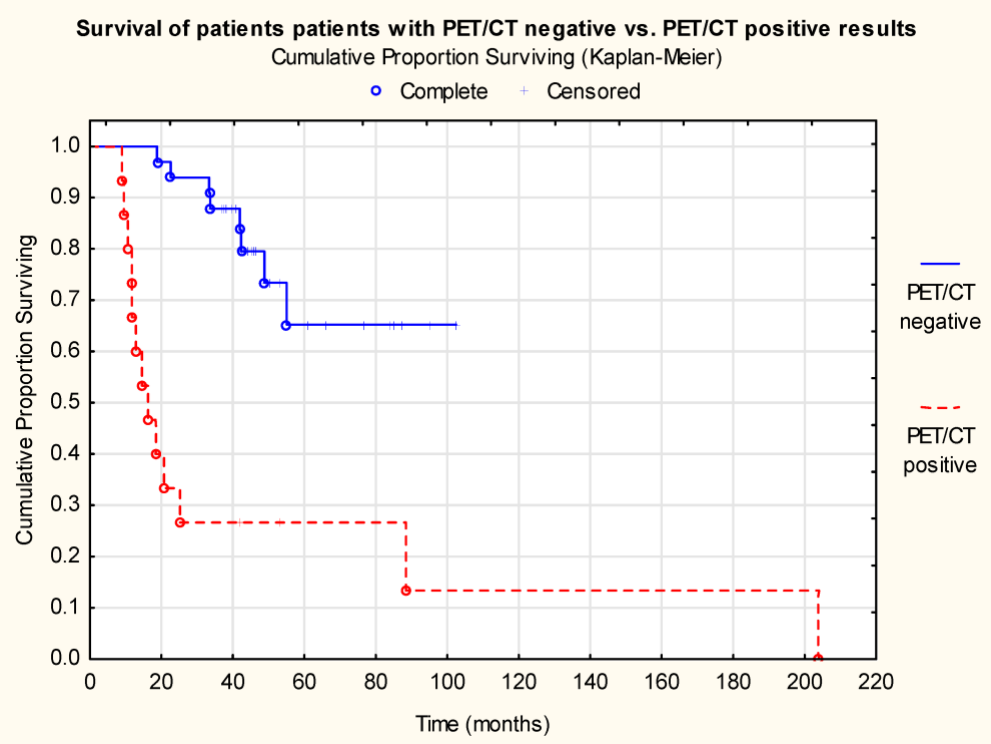


Figure 7: Cumulative proportion surviving (Kaplan-Meier) of patients with PET/CT negative vs. positive results following irradiation (37)

(Log-Rank Test: WW = −9.722, Sum = 20.991, Var = 4.712, Test statistic = −4.478, $p < 0.001$, PET/CT negative with no recurrence in follow up (true negative) n=34, PET/CT positive with nodal positive neck dissection (true positive) n=16, patients at risk n = 50.

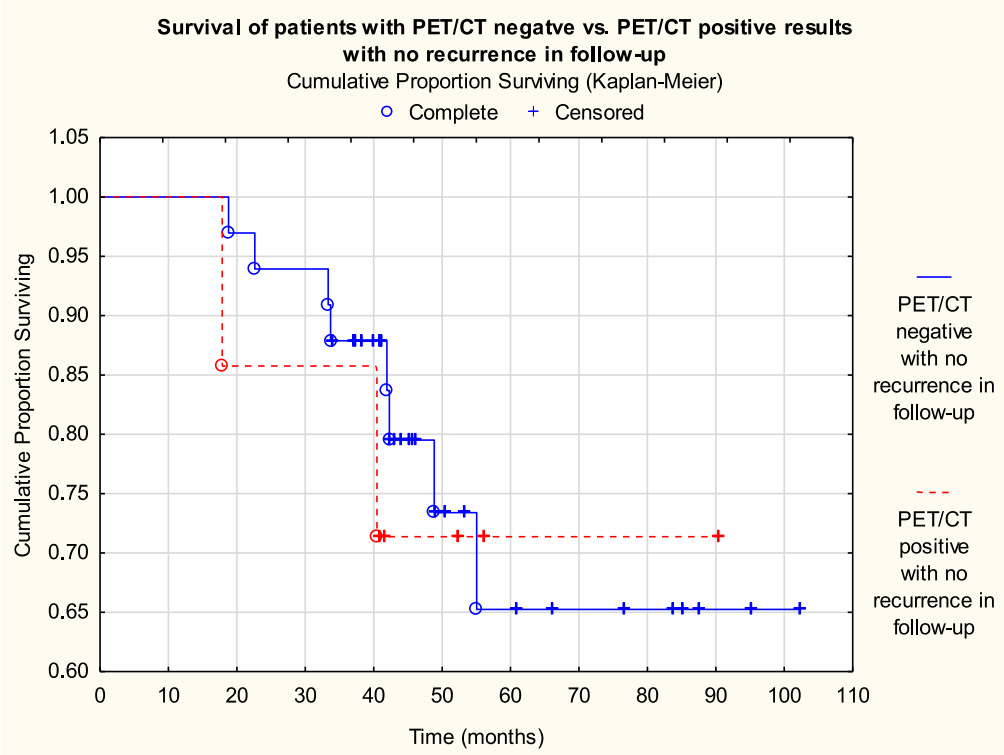


Figure 8: Cumulative proportion surviving (Kaplan-Meier) of patients with PET/CT negative results with no recurrence in follow-up (true negative) and PET/CT positive results with negative neck dissection or no recurrence in follow-up (false positive) (37) (Log-Rank Test: WW = -0.360 Sum = 9.596 Var = 1.4210, Test statistic = -0.302, p = 0.763), n (true negative) = 34, n (true positive) = 16, n (patients at risk) = 50.

Taken together, these results support the alternative hypothesis (H1), demonstrating that post-treatment FDG-PET/CT provides high diagnostic accuracy for the detection of residual nodal disease after definitive chemoradiotherapy. The subgroup analysis also confirmed that diagnostic performance differed according to tumor biology, with more balanced performance in p16-negative than in p16-positive cases.

4.4. Relationship between PET/CT metabolic parameters, PD-L1, and p16/HPV status in OPSCC

Basic characteristics

60 patients with OPSCC were included in the analysis, comprising 32 p16-negative and 28 p16-positive cases. Patients in the p16-positive group were younger at diagnosis (mean 58.2 vs. 62.7 years), and HPV DNA was identified in 24 cases within this subgroup. PD-L1 expression, assessed by TPS (<50% vs. ≥50%) and CPS (<1 vs. ≥1; <20 vs. ≥20), showed a largely similar distribution across p16-negative and p16-positive tumors, with only slight differences between the subgroups. Detailed results are provided in Table 15.

Table 15: Descriptive statistics (38)

		Total	p16-negative	p16-positive
Total patient number		60 (100%)	32 (53%)	28 (47%)
HPV DNA (within p16-positive cases)	negative			2 (7%)
	positive			24 (86%)
	unknown			2 (7%)
Gender	male	32 (53%)	11 (34%)	21 (75%)
	female	28 (47%)	21 (66%)	7 (25%)
Clinical stage	I	13 (22%)	1 (3%)	12 (43%)
	II	13 (22%)	3 (9%)	10 (36%)
	III	9 (15%)	4 (13%)	5 (18%)
	IV	24 (40%)	23 (72%)	1 (4%)
	unknown	1 (2%)	1 (3%)	0 (0%)
T stage	1	4 (7%)	2 (6%)	2 (7%)
	2	22 (37%)	7 (22%)	15 (54%)
	3	12 (20%)	4 (13%)	8 (29%)
	4	22 (37%)	19 (59%)	3 (11%)
	unknown	0 (0%)	0 (0%)	0 (0%)

ECOG status	0	26 (44%)	12 (38%)	14 (50%)
	1	15 (25%)	7 (22%)	8 (29%)
	2	2 (3%)	2 (6%)	0 (0%)
	3	3 (5%)	3 (9%)	0 (0%)
	unknown	14 (23%)	8 (25%)	6 (21%)
Smoking status	Never	14 (23%)	2 (6%)	12 (43%)
	Former	6 (10%)	2 (6%)	4 (14%)
	Current	29 (48%)	24 (75%)	5 (18%)
	unknown	11 (18%)	4 (13%)	7 (25%)
Alcohol consumption	Never/rarely	25 (42%)	10 (31%)	15 (54%)
	Former	6 (10%)	6 (19%)	0 (0%)
	Current	17 (28%)	12 (38%)	5 (18%)
	unknown	12 (20%)	4 (13%)	8 (29%)
TPS	<50%	53 (88%)	30 (94%)	23 (82%)
	≥50%	7 (12%)	2 (6%)	5 (18%)
CPS	<1	14 (23%)	8 (25%)	6 (21%)
	≥1	46 (77%)	24 (75%)	22 (79%)
	<20	44 (73%)	25 (78%)	19 (68%)
	≥20	16 (27%)	7 (22%)	9 (32%)
Age at diagnosis	x<50	11 (18%)	2 (6%)	9 (32%)
	50 ≤x<60	15 (25%)	8 (25%)	7 (25%)
	60 ≤x<70	27 (45%)	19 (59%)	8 (29%)
	70 ≤x<80	6 (10%)	3 (9%)	3 (11%)
	80 ≤x	1 (2%)	0 (0%)	1 (4%)
Remission following full dose irradiation	no	5 (8%)	4 (13%)	1 (4%)
	yes	22 (37%)	8 (25%)	14 (50%)
	criteria not met	33 (55%)	20 (63%)	13 (46%)

Correlation between metabolic PET/CT parameters and PD-L1 expression

In patients with HPV DNA-positive OPSCC, metabolic PET/CT parameters demonstrated a positive association with PD-L1 expression. A consistent relationship with TPS was observed for parameters reflecting the combined primary tumor and nodal disease, including STAGING MTV T+N and STAGING TLG T+N (Spearman's ρ = 0.512 and 0.501, respectively). In cases with CPS >20, the associations remained positive (ρ = 0.491 and 0.441, respectively).

Survival analysis

In univariate Cox regression analysis, TLG of the primary tumor was significantly associated with shorter survival in the overall cohort (p = 0.001, HR (hazard ratio) = 1.002), with similar findings observed in both p16-negative (p = 0.041, HR = 1.001) and p16-positive subgroups (p = 0.011, HR = 1.003).

In the multivariate model, primary tumor TLG remained an independent prognostic factor (p = 0.041, HR = 1.004) after adjustment for p16 status, MTV, and TNM8 tumor stage, while the other variables did not show a significant independent effect.

MTV of the primary tumor showed a less consistent association with survival. Although it reached significance in the overall cohort (p = 0.005, HR = 1.012) and in the p16-positive subgroup (p = 0.003, HR = 1.037), this effect was not observed in p16-negative patients and did not remain significant in the multivariate model (p = 0.163, HR = 0.973). Detailed results of the multivariate analysis are presented in Table 16.

Table 16: Multivariate Cox regression of TLG and MTV (primary tumor), p16 and tumor TNM8 stage. (38) (Test Chi2 = 14.011, df = 4, p = 0.007, N=60)

	Beta	Standard	Beta 95% lower	Beta 95% upper	t-value	Wald	p	Risk ratio	Risk ratio 95% lower	Risk ratio 95% upper
TLG primary tumor (g)	**0.004**	**0.002**	**0.000**	**0.008**	**2.041**	**4.167**	**0.041**	**1.004**	**1.000**	**1.008**
MTV primary tumor (ml, threshold: 2.5)	-0.027	0.020	-0.066	0.011	-1.397	1.950	0.163	0.973	0.937	1.011
p16 0/1 (negative/positive)	-0.618	0.451	-1.502	0.266	-1.370	1.876	0.171	0.539	0.223	1.305
Tumor stage	0.305	0.218	-0.123	0.734	1.397	1.953	0.162	1.357	0.884	2.082

Quartile-based survival analysis showed significant differences in survival for TLG (p = 0.006), whereas no significant survival differences were observed for MTV (Figure 9 A and B). These findings support the prognostic role of TLG over other PET/CT metabolic parameters.

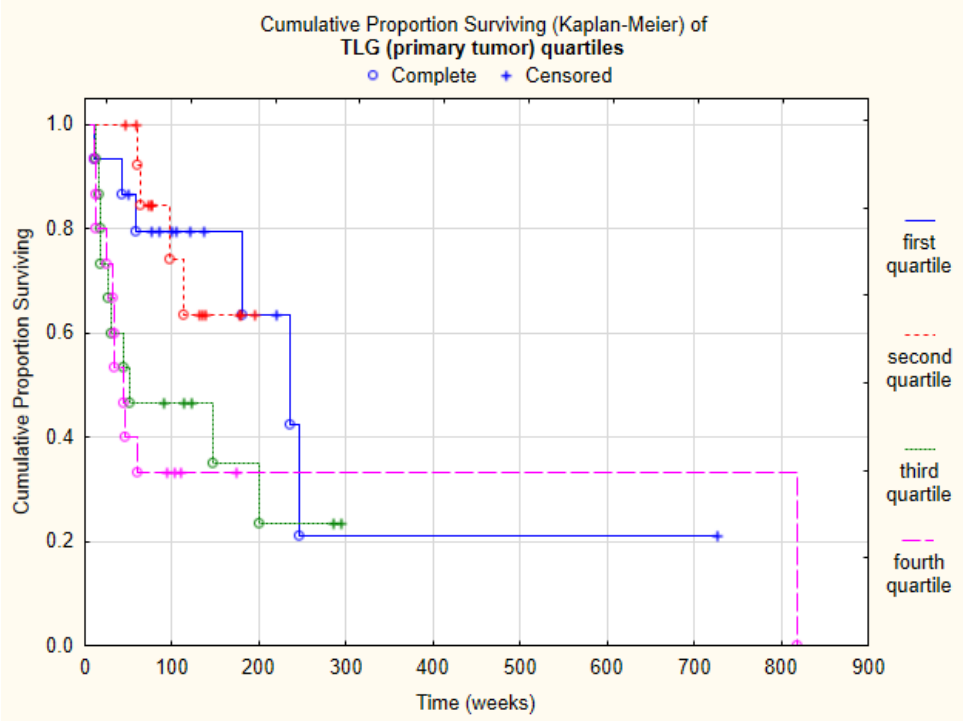


Figure 9 A: Cumulative Proportion Surviving (Kaplan-Meier) of TLG (primary tumor) quartiles on the whole OPSCC population. Chi-square test proved significantly different survival between groups. (38)

first quartile: TLG< 80.2275 g

second quartile: 80.228 g ≤ TLG < 124.975 g

third quartile: 124.975 g ≤ TLG < 280.206 g

fourth quartile: 280.206 g ≤ TLG

(Chi2 = 12.440, df = 3, p = 0.006, N=60)

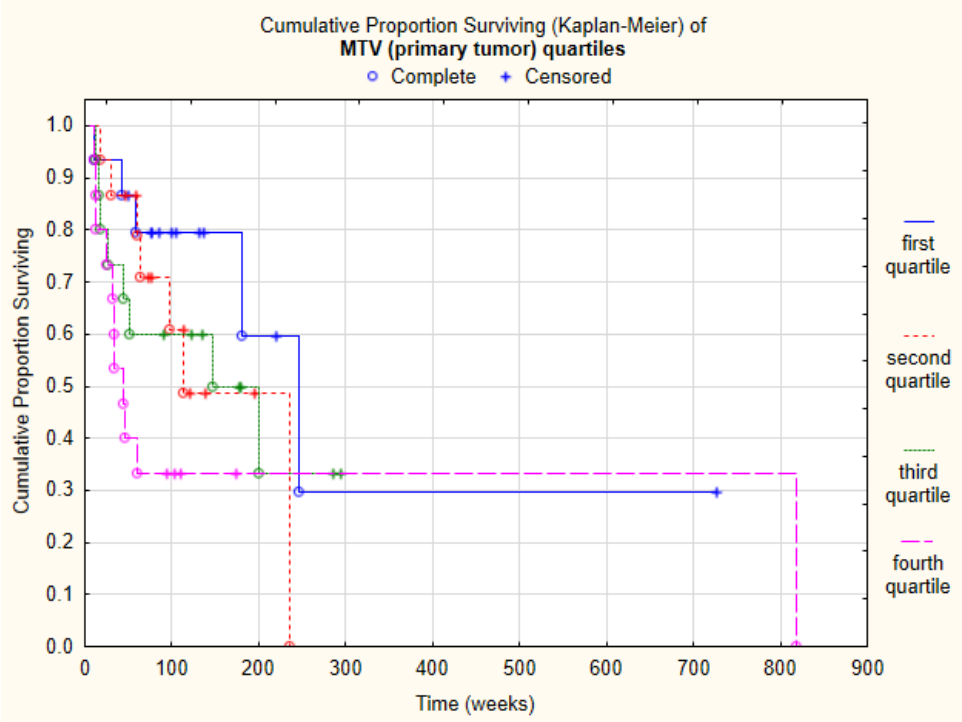


Figure 9 B: Cumulative Proportion Surviving (Kaplan-Meier) of MTV (primary tumor) quartiles on the whole OPSCC population. Chi-square test could not prove significant difference in survival between groups. (38)

first quartile: MTV< 15.62 ml

second quartile: 15.62 ml ≤ MTV < 20.62 ml

third quartile: 20.62 ml ≤ MTV < 39.38 ml

fourth quartile: 39.38 ml ≤ MTV

(Chi2 = 7.499, df = 3, p = 0.058, N=60)

This widely recognized survival advantage was also observed in our study population, with longer survival in p16-positive patients compared to p16-negative cases (log-rank p = 0.038, RR = 0.431). In addition, HPV DNA-positive tumors were associated with better survival (p = 0.005, RR = 0.133).

Association between PET/CT parameters and response to chemoradiation

The predictive value of PET/CT metabolic parameters was evaluated in relation to clinical stage using logistic regression analysis. Among the examined variables, staging MTV of the primary tumor proved to be a significant independent predictor of complete response to chemoradiotherapy ($p = 0.026$; OR = 1.08). Lower MTV values were associated with a higher likelihood of achieving complete remission, independent of tumor stage (Table 17).

No significant predictive effect was observed for the other metabolic parameters, including SUVmax, TLG (primary tumor alone and combined with nodal disease), and MTV incorporating primary tumor and nodal involvement.

Table 17: Logistic regression, where complete remission following full dose irradiation refers to '1', residual disease for '0' in the model. (38)

Final loss: 6.504, Chi2 =12.448, p=0.002, N=26)

	Const.B0	MTV primary tumor (ml, threshold 2.5)	Disease stage (TNM8)
Estimate	-7.750	0.076	1.198
Standard Error	3.710	0.034	0.812
t(23)	-2.089	2.230	1.475
p-value	0.048	0.036	0.154
-95%CI	-15.424	0.005	-0.482
+95%CI	-0.076	0.146	2.878
Wald's Chi-square	4.364	4.973	2.175
p-value	0.037	0.026	0.140
Odds ratio (unit ch)	0.000	1.079	3.313
-95%CI	0.000	1.005	0.617
+95%CI	0.927	1.157	17.784
Odds ratio (range)		1012.909	36.378
-95%CI		1.650	0.235
+95%CI		621781.400	5624.772

Overall, these findings support the alternative hypothesis (H1), showing that PET/CT-derived metabolic parameters are significantly associated with PD-L1 expression and p16/HPV status in OPSCC. Moreover, p16-positive/HPV DNA-positive and p16-negative tumors differed in their volumetric metabolic characteristics, while the association between volumetric PET parameters and PD-L1 expression was evident predominantly in HPV-associated tumors.

5. Discussion

5.1. Patient delay and its clinical significance among head and neck cancer patients

Cancers present with a wide range of symptoms, including general manifestations such as weakness and loss of appetite, as well as site-specific symptoms such as dysphonia and sore throat. In the head and neck region, symptom presentation and clinical relevance are fundamentally linked to function, tumor location, and the involvement of surrounding anatomical structures.

Voice changes in head and neck cancer are typically multifactorial. They may result from disruption of the normal structure and function of the vocal folds, particularly involving the cover-body system, as well as from restricted vocal fold mobility due to tumor invasion into adjacent structures such as the paraglottic space or the cricoarytenoid joint. Neural involvement, especially infiltration of the recurrent laryngeal nerve, may further contribute to impaired phonation (44). Dysphonia was mostly observed in glottic and subglottic laryngeal cancers; however, dysphonia was also associated with prolonged patient delay, which may reflect the more favorable biological behavior and slower progression typically seen in glottic tumors (45).

Referred ear pain in head and neck cancer is related to shared cranial nerve innervation, most often involving the glossopharyngeal and vagus nerves, while the trigeminal nerve is less frequently affected, mainly in oral cavity tumors (46). In our study, ear pain was significantly associated with tumor location, occurring most often in hypopharyngeal cancers.

Sore throat and neck mass were the most frequently reported presenting symptoms in oropharyngeal cancer. Based on p16 status, HPV-positive patients were approximately three times more likely to present initially with a neck mass. This pattern can be explained by the tendency of HPV-associated tumors to develop rapidly enlarging, often cystic cervical metastases (47).

In HPV-positive OPSCC, diagnostic delay is not exclusively patient-related, as several healthcare-associated factors can also contribute, including repeated non-diagnostic FNAC, multiple courses of antibiotic therapy, inappropriate initial surgical management, and insufficient oto-rhino-laryngological evaluation (48). Although this was not the primary focus of our study, it is important to acknowledge these potential contributing factors.

In our study, the mean patient delay in Hungary was 17.57 weeks; in an international context, this is nearly identical to values reported in Canada (17.09 weeks) (49) and in more recent U.S. data (17.03 weeks) (50). In contrast, shorter delays have been described in the United Kingdom (7 weeks for oral cancer and 12 weeks for other head and neck sites) (51) and South India (4.29 weeks) (52). Considerably longer delays have been observed in Brazil (43.48 weeks) (53) and Iran (median 23.05 weeks) (54). Overall, patient delay in the Hungarian population appears to fall within the range reported internationally, with considerable variation across different regions (Table 18).

Table 18: Patient delay times by country (35)

Country	**Patient delay (weeks)**	**Period**	**Region**	**p value**
our data	17.57 (mean)	2012-2017		
Canadian(49)	17.09 (mean)	2017-2018	All HNC	0,598
Brazil(53)	43.48 (median)	2011-2012	All HNC	<0.001
United Kingdom(51)	7.4 (mean)	1995-1996	Oral	0,307
	12.3 (mean)	1995-1996	All HNC ex. oral	0,002
South India (52)	4.29 (median)	2016-2017	All HNC	<0.001
United States(50)	12.67 (not reported)	1993-2004	all HNC	0,001
	17.03 (not reported)	2005-2013	all HNC	0,573
Iran (54)	23.05 (mean)	2004-2006	all HNC	0,002

Survival in head and neck cancers is mainly determined by tumor-related factors, particularly the primary site and stage at presentation (15). This is reflected by the TNM classification, with consistently worse outcomes observed at higher stages. In advanced disease, treatment is typically more intensive, often in the setting of reduced general condition, and is associated with increased toxicity, all of which may further worsen prognosis (55). In line with this, our findings demonstrated differences in stage distribution across tumor localizations, with certain subsites more frequently diagnosed at later stages, further emphasizing the role of tumor site in disease progression and outcome.

In our study, patient delay was not an independent predictor of survival, although it was associated with more advanced stage at diagnosis. Symptom patterns overlapped substantially across anatomical subsites and did not reflect underlying tumor biology, making them unreliable for identifying patients with more favorable prognosis. This likely reflects the heterogeneity of tumor behavior: while some tumors are less aggressive and may remain stable despite longer delays, others may progress rapidly even over a short period. We also observed that the type of presenting symptom was associated with differences in patient delay, with longer delays in patients presenting with voice changes, whereas swallowing-related complaints were associated with earlier medical consultation.

These findings have practical implications for early detection strategies. Early detection efforts may be more effectively directed toward high-risk populations, as focusing on individuals with established risk factors, such as tobacco and alcohol use, may facilitate earlier diagnosis and improve outcomes compared to population-wide screening.

5.2. Accuracy of preoperative staging in the diagnosis of neck metastasis

Accurate assessment of cervical lymph node status plays a central role in treatment selection for head and neck cancer patients. In this study, we evaluated how reliably imaging techniques (CT, MRI, US) and FNAC reflect the true pathological nodal status, in order to better understand how much these modalities can be relied upon in routine clinical staging.

Overall, both imaging modalities and FNAC showed good, but not perfect accuracy. Neither approach reached complete sensitivity, and false-negative findings were observed with both methods: with false-negative rates of 26.1% for imaging and 33.3% for FNAC. These findings are in line with previous reports, which have highlighted similar limitations of conventional imaging, particularly regarding their reduced ability to exclude nodal metastases with confidence (56). Diagnostic performance also depends on lymph node size: while CT and MRI show comparable accuracy for larger lymph nodes, MRI appears to be more sensitive in detecting smaller metastatic nodes (57).

About FNAC, findings from previous studies consistently indicate high specificity and strong positive predictive value, supporting its reliability in confirming nodal metastases. At the same time, its sensitivity remains variable, meaning that negative results cannot reliably exclude metastatic involvement (58-60).

Meta-analytic analysis has compared these modalities, showing that ultrasound-guided FNAC generally provides the most favorable overall diagnostic performance in the detection of cervical lymph node metastases, while conventional imaging techniques demonstrate greater variability, partly due to differences in size and morphological criteria used for nodal assessment (61).

Previous studies comparing the diagnostic performance of CT, MRI, and ultrasound have reported broadly similar accuracy across modalities, in line with our findings; additionally, PET/CT has been shown to provide useful complementary information, particularly in equivocal cases, and may further improve diagnostic performance when used in combination (62-64).

Taken together, our findings indicate that neither conventional imaging modalities nor FNAC alone allow confident exclusion of regional metastatic disease. This limitation is clinically relevant, as occult metastases were present in a considerable proportion of clinically node-negative cases (30.3%).

Considering our null hypothesis, which assumed that preoperative diagnostic methods provide inadequate accuracy for detecting metastatic lymph nodes, based on our data, we accept the null hypothesis.

Accordingly, clinical decision-making should rely on a multimodal approach and adherence to established guidelines. While every effort should be made to achieve the most accurate preoperative staging, a clinically node-negative result alone should not be used to disregard guideline-based recommendations when elective neck dissection is indicated.

5.3. Post-treatment PET/CT accuracy and p16 status

Building on our previous findings, the next question we addressed was how accurately FDG-PET/CT - as the leading functional imaging modality - can confirm or exclude residual disease after chemoradiotherapy in the context of salvage neck dissection. In our experience, this represents a particularly challenging clinical scenario, where accurate decision-making is essential.

Overall, FDG-PET/CT proved to be a reliable tool for the assessment of residual disease in our study. This is in line with previous meta-analyses reporting sensitivity and specificity of approximately 85% and 93%, respectively. Notably, a negative PET/CT finding strongly supports an observation-based approach, thereby reducing the need for salvage neck dissection and its associated morbidity (65). At the same time, we observed a reduction in diagnostic performance in HPV-associated tumors compared to HPV-negative disease (66).

The observed survival patterns were consistent with the pathological reference standard: survival did not differ between true-negative and false-positive cases, whereas patients with true-positive findings had significantly worse outcomes than true-negative individuals.

Although FDG-PET/CT shows a high negative predictive value, additional cytological verification may still be considered in cases with uncertain positive findings. As demonstrated in our earlier work, neither conventional imaging techniques nor FNAC achieve complete sensitivity in the assessment of cervical lymph nodes (36). Accordingly, no single modality can reliably confirm or exclude nodal metastasis, and clinical decision-making should therefore rely on an individualized, multidisciplinary approach, in which FDG-PET/CT plays a central role.

Interpretation of post-treatment PET/CT can be challenging, particularly for clinicians without dedicated expertise in head and neck imaging. Structured reporting systems (e.g., NI-RADS, Hopkins, modified Deauville etc.) may help standardize evaluation and reduce variability, although indeterminate findings remain a persistent limitation. Despite broadly similar performance across these systems, no clear consensus has emerged regarding the optimal approach (67). Integrating structured scoring with clinical judgment and expertise in head and neck imaging may support more appropriate management decisions and help avoid unnecessary surgical interventions (19, 68).

The accuracy of follow-up PET/CT is influenced by several factors, including the timing of imaging, treatment-related tissue changes, inflammatory processes (69) and, as suggested by our findings, p16 status. In p16-positive oropharyngeal cancers, diagnostic performance may be further affected by underlying biological differences, such as increased radiosensitivity and stronger immune-mediated inflammatory response, molecular alterations extending to the surrounding mucosal field (70) and the frequently cystic nature of nodal metastases (47). Previous studies have also reported reduced sensitivity and specificity of FDG-PET/CT in HPV-positive tumors (66).

For this reason, it has been suggested that in p16-positive oropharyngeal cancer, extending the interval between treatment completion and follow-up PET/CT may improve diagnostic reliability without compromising patient safety, as most recurrences occur within the first two years after therapy (71), a period that requires close imaging surveillance. In addition, repeat imaging after initial restaging has been proposed as a potential strategy to reduce false-positive findings (72). In our cohort, however, PET/CT examinations were already performed beyond the commonly used 12-week interval, yet false-positive cases remained, indicating that delaying imaging alone is unlikely to fully resolve this issue.

Based on previously published data in HPV-associated disease, patients with inconclusive PET/CT findings who were managed without early neck dissection had similar survival to those achieving complete remission after chemoradiotherapy (73). These observations support a more cautious approach when considering surgical intervention in this subgroup.

To gain further insight into the causes of discordant results, all cases with mismatched PET/CT findings (false positives and false negatives) were re-examined by a head and neck radiologist with experience in nuclear imaging. The single false-negative case was not entirely unremarkable on initial assessment, and additional follow-up imaging had already been suggested at that time to clarify the suspicious features. In five of the seven false-positive cases, suspicion of residual disease was not based on FDG uptake on PET but rather on morphological abnormalities seen on CT. In one patient, imaging was performed relatively early after treatment (just beyond 11 weeks), which may have contributed to diffuse - inflammatory - uptake, while in another case, retrospective evaluation confirmed focal uptake consistent with residual disease. Overall, discordant

findings were evenly distributed, with four cases occurring in p16-positive oropharyngeal carcinoma and four in p16-negative tumors.

One promising strategy in diagnostically uncertain cases is the assessment of circulating tumor HPV DNA (ctHPVDNA). According to a recent systematic review and meta-analysis, ctHPVDNA demonstrated high diagnostic accuracy for the detection of residual or recurrent disease in HPV-positive OPSCC (sensitivity: 0.86; specificity: 0.96), while PET/CT showed overall weaker diagnostic performance (sensitivity: 0.89; specificity: 0.83). These findings suggest that the combined use of ctHPVDNA and PET/CT may be the most advantageous approach in post-treatment surveillance. In cases of equivocal PET/CT findings, a negative ctHPVDNA result may support close observation rather than immediate invasive intervention, whereas persistently detectable or increasing ctHPVDNA levels may indicate the need for intensified follow-up and earlier imaging-based reassessment to guide possible salvage treatment (74).

In summary, this patient population - particularly those with inconclusive findings - may benefit from evaluation in specialized tertiary centers, where multidisciplinary expertise can support both restaging and clinical decision-making (16).

In such settings, management aims to balance oncologic safety with the best possible quality of life. However, in line with current national recommendations (75), priority is often given to oncologic control, even if this approach carries a risk of overtreatment. Reflecting this principle, our clinical practice favored a more cautious strategy in uncertain cases, which led to a higher number of neck dissections that may not have been necessary.

5.4. Relationship between PET/CT metabolic parameters, PD-L1, and HPV status in OPSCC

FDG PET/CT has become an integral part of the staging in oropharyngeal cancer, with increasing evidence supporting the prognostic relevance of volumetric metabolic parameters. In particular, based on our findings, total lesion glycolysis reflects tumor burden and may help identify patients with higher-risk disease, who are less likely to be suitable candidates for treatment de-escalation.

At the same time, the growing use of immune checkpoint inhibitors has shifted attention toward immune-related prognostic and predictive factors. In this context, understanding the relationship between tumor metabolism and immune activity has become increasingly important. In our study, a correlation was observed between metabolic PET/CT parameters, such as TLG and MTV, and PD-L1 expression (as reflected by TPS) in HPV-associated oropharyngeal cancers, which may provide additional insight into tumor biology and contribute to improved risk stratification.

PD-L1 expression has been increasingly recognized as a clinically relevant biomarker, especially in patients receiving immune checkpoint inhibitor therapy, where higher expression levels have been associated with improved survival outcomes in head and neck cancers (76).

Several studies have also explored the link between tumor metabolism and immune-related features. Yun Chen et al. demonstrated that PD-L1/PD-L2 expression correlates with FDG PET-derived textural parameters, suggesting that metabolic heterogeneity may reflect underlying immune activity (77). Similarly, Takahashi et al. reported an association between volumetric PET parameters, including MTV and TLG, and PD-L1 expression, supporting the concept that metabolic tumor burden is linked to immune regulation (78). This concept suggests that increased glycolytic activity may be accompanied by upregulation of immune checkpoint pathways, reflecting a biologically more active tumor microenvironment (32, 79). In line with this, our findings further support this relationship, as a positive correlation was observed between volumetric PET/CT parameters -TLG and MTV- and PD-L1 expression (both TPS and CPS >20) in HPV-associated OPSCC. This relationship, however, was not evident in p16-negative tumors, pointing toward potentially different regulatory pathways of PD-L1 expression in this subgroup. Taken together, these findings highlight the potential clinical utility of

PET/CT-derived volumetric biomarkers in the non-invasive assessment of tumor immune status. PET imaging may offer an indirect approach to estimating PD-L1 expression, thereby supporting patient selection for immunotherapy, particularly in settings where tissue-based assessment is limited or repeated evaluation is required.

These findings also raise the possibility that metabolic imaging could complement tissue-based PD-L1 assessment, especially when biopsy material is limited or dynamic evaluation is required.

Beyond their association with immune-related features, metabolic PET/CT parameters also showed prognostic relevance in our study, with higher staging MTV and TLG associated with worse survival. This finding is consistent with previous reports, including meta-analyses demonstrating that elevated volumetric PET parameters are linked to poorer survival outcomes in head and neck cancers (80). As these measurements are readily available in routine clinical practice, they may support risk stratification and contribute to clinical decision-making (81).

The MTV of the primary tumor also proved to be a relevant predictor of treatment response, as it independently correlated with complete remission following CRT. This observation is consistent with previous evidence highlighting the role of tumor volume in treatment outcomes in advanced HNSCC (82). In contrast, a recent review suggests that the predictive value of metabolic parameters may depend on HPV status, with higher pre-treatment, combined primary and nodal disease MTV and TLG associated with poorer outcomes mainly in HPV-negative cases, while findings remain less consistent in HPV-positive tumors (42). However, based on our findings, primary tumor MTV may help identify patients more likely to benefit from CRT.

HPV-positive and HPV-negative OPSCCs show distinct microRNA expression patterns, reflecting divergent molecular characteristics. Combining these with metabolic parameters and immunological biomarkers may improve patient management (70).

6. Conclusions

Taken together, the studies included in this dissertation show that accurate evaluation of head and neck cancers depends not only on imaging itself, but also on choosing the appropriate modality for the given clinical situation and understanding the strengths and limitations of each method. Conventional imaging remains essential in everyday practice, yet functional imaging is gaining increasing importance, not only in treatment response assessment but also in improving our understanding of tumor behavior and biological heterogeneity. This is particularly true in HPV-associated oropharyngeal squamous cell carcinoma, where the generally better prognosis is accompanied by specific difficulties in neck management and posttreatment assessment.

The study on patient delay adds an important clinical aspect to these findings. Although Hungarian patients with head and neck cancer often waited a relatively long time before seeking medical attention, delay itself did not significantly influence survival. This was also reflected in the differences observed between p16-positive and p16-negative oropharyngeal cancers, in which symptom pattern and survival differed despite the lack of a significant difference in delay duration. Instead, the results suggest that clinical outcome is more strongly determined by tumor localization, stage, and biological behavior than by the duration of symptoms alone. At the same time, the long delay observed in this population highlights the importance of patient education and greater awareness in primary care for earlier recognition of suspicious symptoms.

In the preoperative setting, the main conclusion is clear: neither conventional imaging nor FNAC is reliable enough to exclude cervical metastases with confidence. Negative preoperative findings alone therefore do not justify omission of neck dissection when it is otherwise indicated by current guidelines. In the posttreatment setting, FDG-PET/CT proved most useful for excluding residual nodal disease. Its negative results supported observation with a high degree of confidence, whereas positive or equivocal findings, especially in p16-positive disease, were more difficult to interpret because posttreatment changes could mimic residual tumor and lead to overtreatment. In such cases, expert head and neck radiological review, standardized interpretation, and, when needed, repeat imaging are essential.

The PET/CT metabolic study showed that imaging may also provide information beyond anatomical staging and restaging. In OPSCC, volumetric PET/CT parameters were

associated with clinically relevant features of tumor behavior. Primary tumor TLG was linked to survival, while primary tumor MTV was associated with complete remission after chemoradiotherapy. In HPV-associated disease, volumetric parameters also correlated with PD-L1 expression, suggesting a relationship between metabolic activity and the tumor immune microenvironment. This indicates that PET/CT may help connect imaging findings with prognosis and tumor biology in a non-invasive way.

Overall, the results of this dissertation support a more integrated interpretation of imaging in head and neck oncology. Imaging should be considered together with the clinical setting and the biological characteristics of the tumor, rather than as an isolated diagnostic result. This approach may improve treatment selection, help avoid unnecessary procedures, and support more individualized decision-making in patients with head and neck cancer.

7. Summary

Introduction: Accurate diagnosis is essential in HNSCC, particularly in HPV-associated OPSCC, which differs from HPV-negative disease in clinical behavior and imaging characteristics. This dissertation evaluated pre- and posttreatment imaging in HNSCC, focusing on nodal assessment, PET/CT-derived metabolic parameters in relation to p16/HPV status and PD-L1 expression, and the clinical significance of patient delay.

Methods: Four studies were included. Patient delay was analyzed retrospectively in 236 patients with squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx. Preoperative imaging and FNAC were compared with histopathology in 62 surgically treated patients. Posttreatment FDG-PET/CT was prospectively evaluated in 58 node-positive HNSCC patients after chemoradiotherapy. Finally, 60 newly diagnosed OPSCC patients were analyzed for associations between PET/CT-derived metabolic parameters, PD-L1 expression, p16/HPV status, survival, and treatment response.

Results: Hungarian HNSCC patients showed a relatively long patient delay (mean 17.57 weeks), but delay did not significantly influence survival. In OPSCC, delay duration was similar in p16-positive and p16-negative cases, whereas symptoms and survival differed. Preoperative imaging and FNAC were clinically useful but limited in excluding nodal metastases, with false-negative rates of 26.1% and 33.3%. Posttreatment FDG-PET/CT showed high overall diagnostic accuracy, especially for ruling out residual nodal disease (NPV 97%); however, in p16-positive OPSCC, false-positive findings were more frequent, resulting in a PPV of 43%. In OPSCC, primary tumor TLG was independently associated with survival, primary tumor MTV was associated with complete remission after chemoradiotherapy, and in HPV-associated disease, volumetric PET parameters correlated positively with PD-L1 expression.

Conclusion: Patient delay was relatively long, supporting the need for patient education and earlier symptom recognition, although it did not independently determine survival. Conventional preoperative imaging and FNAC cannot reliably exclude cervical metastases, whereas posttreatment PET/CT is most valuable for ruling out residual nodal disease, with positive findings in HPV-associated OPSCC requiring caution. PET/CT-derived metabolic parameters may provide prognostic, predictive, and immune-related information, supporting more accurate risk stratification and individualized treatment planning.

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9. Bibliography of the candidate's publications

9.1 Publications related to the dissertation

- Danos K, Horvath A, Kristof E, Uri I, Besenczi B, Prekopp P, et al. Post-Radiation PET Shows Higher Diagnostic Accuracy in HPV-Negative Head and Neck Cancers. Cancers (Basel). 2026;18(8). http://doi.org/10.3390/cancers18081237
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9.2 Publications not related to the dissertation

- Besenczi, B., Horváth, A., Uri, I., & Dános, K. (2026). The effect of the COVID-19 pandemic on TNM status among head and neck cancer patients in Hungary. PATHOLOGY AND ONCOLOGY RESEARCH, 32. http://doi.org/10.3389/pore.2026.1612298
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