Diploma thesis

Is the 8th version of tumor classification for p16-positive and p16-negative oropharyngeal cancers of adequate quality

Submitted by

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Declaration in Lieu of Oath

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 04.11.2021 *Anna Strasser eh*

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Abbreviations

A.	Arteria
AJCC	American Joint Committee on Cancer
BSC	Best supportive care
CI	Confidence interval
CR	Complete remission
CRT	Chemoradiotherapy
CT	Computed tomography
cTNM	Clinical Version of TNM
DFS	Disease free survival
DNA	Deoxyribonucleic acid
E6	early gene 6
E7	early gene 7
ECOG	Eastern Cooperative Oncology Group, the ECOG score describes the physical performance of cancer patients
e.g.	For example
ENE	Extra nodal Extension
ENT	Ears, nose, and throat
FFPE	Formalin-fixed, paraffin-embedded tissue
Fig.	Figure
Gl.	Glandula
HNSCC	Head and Neck squamous cell carcinoma
HPV	human papilloma virus
IARC	International Agency for Research in Cancer
ICON-S	International Collaboration on Oropharyngeal cancer Network for Staging
IHC	immunohistochemistry
IMRT	Intensity-Modulated Radiation Therapy
ISH	In situ hybridization
M	M category in the TNM system stands for (distant) metastasis
M.	Musculus
m^2	Square meter
Mal.	Malignoma
Meta.	Metastasis

mg	Milligram
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
N	N category in the TNM system stands for (lymph) nodal extension
N.	Nervus
NCCN	National comprehensive cancer network
Neg.	Negative
OP	Operation
OPC	Oropharyngeal carcinoma
OPSCC	oropharyngeal squamous cell carcinoma
OS	Overall survival
p16	p16-Protein
PAR	population attributable risk
PD	Progressive disease
PD-L1	programmed death-ligand 1
PET	Positron emission tomography
PR	Part remission
Pos.	Positive
pTNM	Pathological Version of TNM
R.	Ramus
RB	retinoblastoma protein
Rec.	recurrence
RR	Relative Risk
RT	Radiotherapy
rtPCR	reverse transcriptase polymerase chain reaction
SCC	Squamous cell carcinoma
SD	Steady disease
T	T category in the TNM system stands for tumor extension
TB	Tumor Board, stands for Head and Neck Tumor Board
TNM	International Tumor staging system, each letter should describe the extent of the tumor/ where it has spread
UICC	Union international contre le cancer
V.	Vena

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Zusammenfassung

Hintergrund: Die Evaluierung der Qualität und des prognostischen Wertes der 8. Version der UICC TNM Klassifikation im Vergleich zur 7.Version in Bezug auf Humane-Papillomavirus-(HPV)-assoziierte Oropharynxkarzinome ist Gegenstand aktueller Forschung.

Methoden: Krankenakten von Patienten*innen einer tertiären Gesundheitsversorgung in der Steiermark wurden retrospektiv analysiert. Eingeschlossen wurden alle geeigneten Patient*innen bei denen im Zeitraum von 2015-2018 erstmals ein Oropharynxkarzinom diagnostiziert wurde und die primär an der tertiären Gesundheitseinrichtung, entweder operativ oder mittels konservativer Radiochemotherapie, behandelt wurden. Der HPV-Status der Tumore wurde mittels einer p16-Testung ermittelt. Um einen korrekten Vergleich zu ermöglichen, wurden alle Patient*innen entsprechend der 7. Version (TNM7) und der 8. Version der UICC TNM Klassifikation (TNM8) in Tumorstadien eingeteilt. Das Gesamtüberleben und das krankheitsfreie Überleben wurden mittels der Kaplan-Meier Methode errechnet.

Ergebnisse: Insgesamt konnten 178 Patient*innen inkludiert werden, deren Tumore in 49,4% p16-positiv und 44,4% p16-negativ waren. Bei 6,2% der Tumore konnte der p16-Status retrospektiv nicht aus den Krankenakten erhoben werden, weshalb sie für Analysen basierend auf dem p16-Status exkludiert wurden.
Je nach angewendeter Version der UICC TNM Klassifikation konnte in der Gruppe der p16-positiven Oropharynxkarzinome eine bemerkenswerte Verlagerung zwischen den Tumorstadien beobachtete werden. Als Stadium I wurden n=4 (TNM7) bzw. n=39 (TNM8) Patient*innen klassifiziert, n=7 (TNM7) bzw. n=19 (TNM8) als Stadium II, n=8 (TNM7) bzw. n=27 (TNM8) als Stadium III. Wurden die Patient*innen entsprechend TNM7 klassifiziert, waren n=59 dem Stadium IVA zugehörig, n=7 dem Stadium IVB und n=3 dem Stadium IVC, während mit TNM8 nur n=3 Patient*innen als Stadium IV klassifiziert wurden.
Die Gesamtüberlebenskurven zeigten, wenn TNM7 angewendet wurde, dass Patient*innen mit p16-positiven Tumoren in Stadium III und Stadium IV ein besseres Gesamtüberleben hatten als die Patient*innen in Stadium I und II. Diese Fehldarstellung wurde mit TNM8 berichtigt, hier zeigte sich bei Stadium I Tumoren eine 3-Jahres-Überlebensrate von 94%, bei Stadium II lag sie bei 85% und bei Stadium III bei 81%. Der Vergleich aller Stadien

gemeinsam zeigte einen signifikanten Unterschied ($p<0,001$). Wurden die einzelnen Stadien jedoch paarweise verglichen, unterschieden sich nur Stadium III und IV statistisch signifikant ($p<0,001$).
Die Analyse des krankheitsfreien Überlebens von Patient*innen mit p16-positiven Tumoren führte zu ähnlichen Ergebnissen. So war das krankheitsfreie Überleben, wenn TNM7 angewendet wurde, in Stadium III besser als in Stadium I. Wurde jedoch TNM8 verwendet, zeigte sich eine 3-Jahres Rate des krankheitsfreien Überlebens von 80% bei Stadium I Tumoren, bei Stadium II Tumoren lag sie bei 59% und bei Stadium III Tumoren bei 63%. Auch bei der Analyse des krankheitsfreien Überlebens zeigte sich der einzige statistisch signifikante Unterschied zwischen Stadium III und IV in dieser Kohorte ($p<0,001$).

Schlussfolgerung: Die 8. Version der UICC TNM Klassifikation ist hinsichtlich des prognostischen Wertes für p16-positive Oropharynxkarzinome eine Verbesserung im Vergleich zu seinem Vorgänger. Weitere Überarbeitungen könnten die Klassifikation und Prognose insbesondere für die Stadien II und III verbessern.

Abstract

Background: Evaluation of the quality and the predictive value of the 8th UICC TNM compared to the 7th UICC TNM staging manual regarding oropharyngeal squamous cell carcinoma associated with human papillomavirus (HPV) are currently active fields in research.

Methods: Medical records of patients of a tertiary care center in Styria were retrospectively analyzed, included all eligible patients initially diagnosed with OPSCC from 2015 through 2018. These patients were primarily treated either surgically or with (chemo)radiotherapy or both at a single comprehensive cancer center. HPV status was determined by p16 testing. All patients were retrospectively staged according to both the 7th Edition (TNM7) and 8th Edition (TNM8) of UICC TNM classification for comparison. Overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan-Meier method.

Results: A total of 178 patients were included; 49,4% of tumors were p16-positive, 44,4% were p16-negative, and in 6,2% the p16-status could not be determined in the medical records. Therefore, for analyses based upon the p16-status, that 6,2% of undefined patients were not considered.

A notable shift in the TNM stage was observed for patients with p16-positive OPSCC, comparing 7th Edition vs. 8th Edition of UICC TNM classification: N=4 (TNM7) vs. n=39 (TNM8) patients were staged as stage I, n=7 (TNM7) vs. n=19 (TNM8) as stage II, n=8 (TNM7) vs. n=27 (TNM8) as stage III. According to TNM7, n=59 patients have been classified as stage IVA, n=7 as stage IVB, and n=3 as stage IVC, whereas only n=3 patients were left to be classified as stage IV with TNM8.

When applying TNM7, the OS functions showed that patients with p16-positive OPSCC with stage III and IVA had a better OS than those with stage I and II. This misrepresentation changed when TNM8 was applied. The 3-year OS-rate for stage I tumors was 94%, for stage II 85% and for stage III 81%. When comparing overall stages, TNM8 showed a significant difference ($p<0,001$). This changed when adjacent stages were compared pairwise, only stage III and IV differed significantly ($p<0,001$).

The analysis of DFS of patients with p16-positive OPSCC led to similar results. The DFS of stage III was superior to the one of stage I in TNM7. When applying TNM8 the 3-year DFS-rate for patients with a stage I carcinoma was 80%, for stage II tumors 59% and for stage III

tumors 63%. Again, the only statistically significant difference within this study cohort could be found between stage III and IV ($p<0,001$).

Conclusion: The 8th Edition of UICC TNM classification has improved compared to its predecessor regarding the predictive value for p16-positive OPSCC. Further adaption may help to improve the classification and prognosis even more, particularly for stage II and III.

1 Introduction

1.1 Anatomy

1.1.1 Pharynx

The Pharynx is located anterior to the cerebral vertebral column, with a total length of 12-15 cm. It is basically the connection between the oral cavity and the esophagus. In clinical practice, the pharynx is divided into three different parts (1).

Firstly, there is the nasopharynx, which is the most cranial part of the pharynx. Its extension reaches from the skull base to the soft palate. The nasopharynx relates to the nasal cavity.

Secondly, the oropharynx, which is the most relevant for this work. This part of the pharynx extends from the soft palate to the top edge of the epiglottis. The oropharynx is directly connected to the oral cavity via the isthmus of fauces. (2)

Clinically relevant are the precise limits between the oral cavity and the oropharynx, as tumors of the oral cavity tend to metastasize different than tumors of the oropharynx resulting in different prognoses. These limits are:

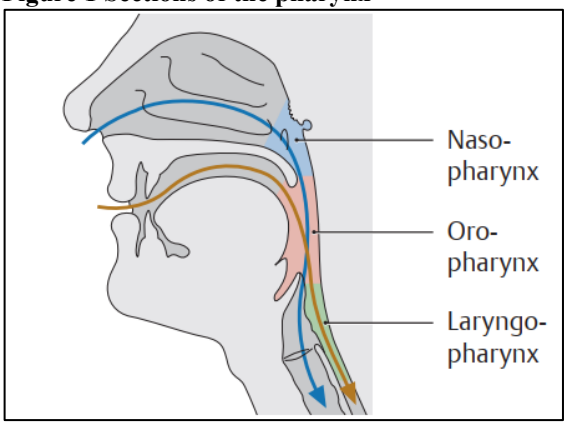
- Superior: the shift between the hard palate (still part of the oral cavity) and the soft palate (already part of the oropharynx)
- Lateral: the palatoglossal arch
- Inferior: the vallate papillae of the tongue (3)

An essential part of the oropharynx is the tonsillar fossa, located at both sides between the palatoglossal and the palatopharyngeal arch. These are not the only tonsils one can find in the oropharynx; the so-called lingual tonsils are located in the base of the tongue (2), which is the oropharyngeal part of the tongue being dived from the oral part by the vallate papillae. (3)

Moreover, the palatine and the lingual tonsils are part of the group of lymphoid tissue in the pharynx, often referred to as the so-called Waldeyer´s tonsillar ring, named after the famous anatomist. Other tonsils that belong to this group are the pharyngeal and tubal tonsils located in the Nasopharynx and, therefore, less relevant for this work. (2)

Thirdly, everything beneath the top edge of the Epiglottis to the cricoid cartilage belongs to the larynx or hypopharynx.

Figure 1 Sections of the pharynx



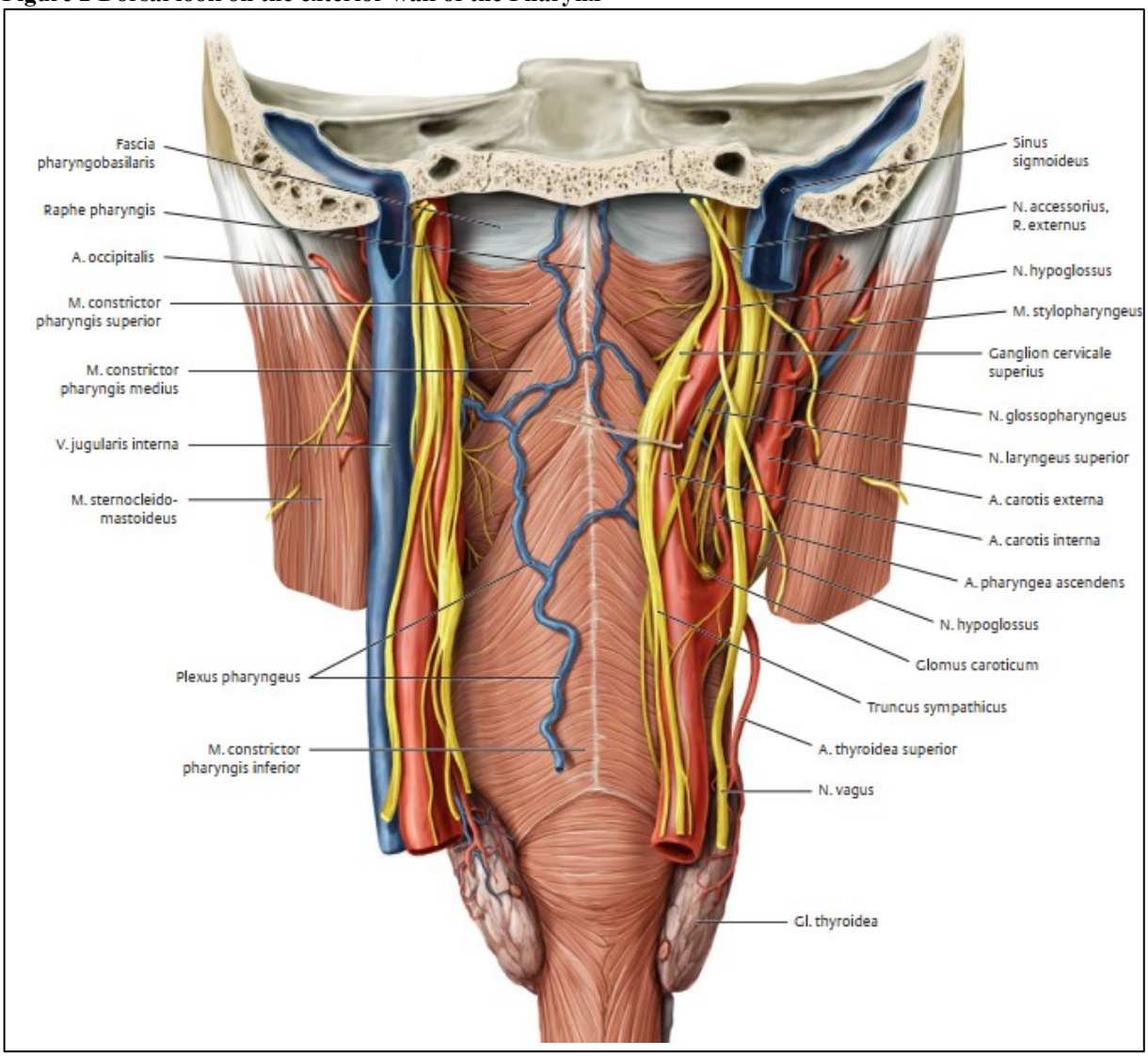
Schünke et al. (4) p.202
This figure shows the three different sections of the pharynx and how air (blue) and food (brown) cross in the oropharynx.

Knowing the pharynx's anatomy and topography is fundamental to every head and neck surgeon, as the pharynx has close connections to vessels and nerves, which are essential to life. For example, lateral of the pharynx run the common carotid, the internal jugular vein, the sympathetic trunks, and the vagus nerve. (2)

Moreover, the knowledge of which vessels supply the pharynx itself is indispensable for surgeons. During surgery, massive bleeding can happen, and knowledge of vessels' anatomy is essential to stop hemorrhage and ligate or reconstruct bleeding vessels. The vessels that subserve the pharynx itself are the pharyngeal branches of the ascending pharyngeal artery, also the ascending palatine artery (both vessels are descendants of the external carotid artery) and the pharyngeal branches of the inferior thyroid artery (descendant of the subclavian artery), the latter supply mainly the hypopharynx. Venous drainage happens via the pharyngeal plexus and the pterygoid plexus, which drain into the internal jugular vein.

Relevant for the neural innervation of the pharynx are the glossopharyngeal nerve, the vagus nerve, and the sympathetic trunk. The glossopharyngeal nerve innervates the muscles of the pharynx and is also responsible for the sensible and sensory innervation of the tongue (2). Patients can lose taste and develop difficulties swallowing if an injury happens to the above-mentioned neural structures during surgery. Another nerve that can be harmed during an oropharyngeal surgery is the hypoglossal nerve, which innervates the tongue motorically. An injury to this nerve causes reduced and asymmetrical motility of the tongue, leading to trouble in speaking and swallowing. (5)

Figure 2 Dorsal look on the exterior wall of the Pharynx



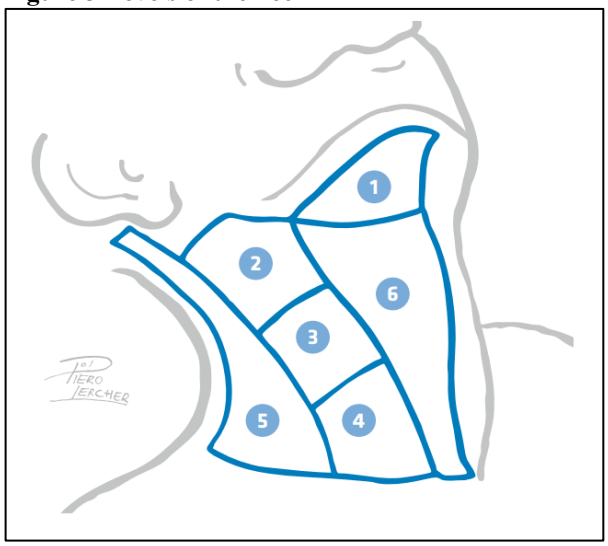
Weiglein.(2) p.829
This figure shows the topography of the exterior wall of the pharynx when being looked at from dorsal and how the pharynx is closely connected to the essential vessels and nerves of the neck.
Abbreviations: A.: Arteria, V.: Vena, R.: Ramus, N.: Nervus, M.: Musculus, Gl.: Glandula

1.1.2 Neck-Level

Around 300 lymph nodes can be found in the human neck. Cervical lymph nodes are subdivided into six levels (I-VI) following specific landmarks and anatomic features. For example, the submandibular nodes are in Level Ib, adjacent to the mandible and the two bellies of the digastric muscle, defined as the submandibular triangle. This terminology helps surgeons to communicate about the individual surgical extensions of neck dissections (3). Moreover, the cervical levels are essential in follow-up care, as radiologists may distinguish between possible residual lymph nodes, lymph node metastasis, or soft tissue metastasis in the former surgical area.

Besides, the knowledge of the lymph node levels is of immense interest for diagnosing. Depending on the lymph node levels, surgeons can conclude which organ is affected or vice versa. For instance, the submandibular lymph nodes drain the oral cavity, the nose's outer parts, and a part of the face. Tumors of the oropharynx are known to affect level II, III, and IV regularly. (6)

Figure 3 Levels of the neck



Modified, Thurnher et al. (3) p. 371
This figure shows the levels of the neck.

1.2 Pathology of oropharyngeal carcinomas

1.2.1 Localization

The oropharynx can be divided into four oncological subgroups. Carcinomas can be found in the tonsils, the base of the tongue, the soft palate (and the uvula), and the posterior wall of the pharynx. (3)

1.2.2 Histology

The type of epithelium found in the oral cavity and the oropharynx is a non-keratinized stratified squamous epithelium. (2) As this is the dominant epithelium in this region, the conclusion can easily be drawn that squamous cell carcinomas (SCC) are also dominant in this area. To be specific, more than 90% of the head and neck cancers are SCCs, but there can also be found other types of cancers, such as sarcomas, melanomas, lymphomas, and adenocarcinomas. (3)

The palatine tonsil stands out of this group because of its unique histological structure. It is covered with non-keratinized stratified squamous epithelium and contains many immune defense cells such as lymphocytes. Therefore, about 75% of the cancers of the tonsils are SCC, about 2% Hodgkin- and 15% Non-Hodgkin Lymphomas. (7)

1.2.3 Etiology and pathogenesis

There are several predisposing risk factors for oropharyngeal squamous cell carcinoma (OPSCC). To start with, OPSCC is related to alcohol and tobacco consumption; those two substances have been often excessively misused over decades. In addition, alcohol amplifies the impact of smoking as it functions as a solvent for the carcinogens that tobacco contains. There are about 70 cancer-causing substances found in tobacco (3). Hashibe et al. (8) showed in their pooled analysis that the *'population attributable risk (PAR) for tobacco or alcohol was 72% (95% CI: 61-79%)'* ((8) p.545), meaning that people without a history of excessive drinking or smoking have a 72% smaller risk of getting an OPSCC than those who have.

Other approved risk factors are infections with high-risk human papillomaviruses. This correlation has been suspected for a long time; back in 1983, Syrjänen et al. (9) have published a study claiming that HPV could be involved in the carcinogenesis of oral cancers. Since then, a lot of evidence has been collected, and 26 years later, the hypothesis was officially confirmed. In 2009 the International Agency for Research in Cancer (IARC) (10) stated that Human Papilloma Virus type 16 (HPV-16) is carcinogenic to humans. In addition, the IARC confirmed the pathogenesis of how HPV influences the development of OPSCC, such as '*cell Immortalization, genomic instability, inhibition of DNA damage response, anti-apoptotic activity*'((10) p.321). OPSCC are often localized in the lingual tonsil and the palatine tonsils, which are lymphoid rich and part of the oral immune defense. Therefore, it seems somewhat paradoxical, that especially those lymphoid rich organs are affected by HPV, resulting in carcinomas. According to Lyford-Pike et al. (11) the deep crypts of the tonsils are an '*immune-privileged'* (p.1734) site for viral infections, encouraging the persistence of the virus and resulting in malignant transformation of the cells. This is because the deep crypts express PD-L1 (programmed death-ligand 1), which decreases the function of virus-specific T-cells. (11)

1.2.4 Incidence

Head and neck cancers represent 6% of all cases of cancer in global comparison and estimations imply that every year there are about 500.000 new cases detected. (3) According to their latest data, STATISTIK AUSTRIA (12) claims that the incidence of head and neck cancers in Austria is 14.4 (new cases/100.000 habitants) and there is a 1% risk of developing one before the age of 75. There are no published numbers on the incidence of OPSCC exclusively, but on the prevalence of HPV in OPSCC. In a systematic review published in 2013, Mehanna et al. (13) claim that the '*overall pooled prevalence in patients with OPC was 47.7% (95% CI, 42.9–52.5)*' (p.751). Their results show an increase over time in

prevalence in both North America and Western Europe, starting with *'40.5.% (95% CI, 35.1–46.1)'* ((13) p.747) before 2000 and resulting in '*72.2% (95% CI, 52.9–85.7)'* ((13) p.747) after 2005 onward. According to this review, this increase is not due to better sensitivity or testing techniques, instead, the prevalence of HPV in OPSCC is rising for real.

1.3 Diagnosis

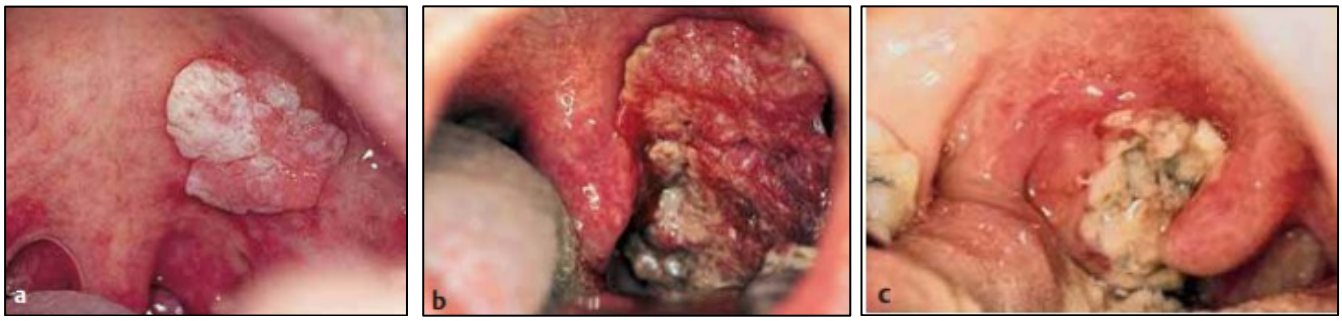
1.3.1 Symptoms

Usually, patients with OPSCC are diagnosed with an advanced stage of their disease because there are few/ non-specific symptoms in early-stage disease OPSCC. Later patients present themselves with symptoms like odynophagia, dysphagia, trismus, bad breath odor, restrictions in the movement of the tongue or painless swollen lymph nodes. The latter is often the first symptom that patients recognize and why they see a doctor. (7)

1.3.2 Medical history and examination

Patients are being asked about the symptoms mentioned above and the so-called "B-symptoms"; unexpected weight lost, fever or night sweats. Moreover, past, or current smoking history and the consumption of alcohol are of particular interest. After a thorough medical history taking, patients are being physically examined. Typically, the tonsils are enlarged at one side, possibly even ulcerous lesions are visible. Lesions of the tongue base are more challenging to spot with the naked eye, but devices like a flexible endoscope can help reveal them. Also, the examiner palpates the patient´s neck for swollen lymph nodes and the oral cavity, if the pharyngeal reflex of the patient does not interfere with that. (7)

Figure 4 Carcinomas of the soft palate and the tonsils



Arnold et al. (7) p.357. (Translated from German) These figures show:

a) Exophytic growing squamous cell carcinoma of the soft palate above the left tonsil.
b) Extensive carcinoma of the left tonsil spreading to the soft palate, causing odynophagia and otalgia.
c) Extensive carcinoma of the right tonsil with vast spreading to the surrounding tissue, causing odynophagia, painless.

1.3.3 Imaging

To decide how to treat an OPSCC physicians in charge need detailed information about the extent of the tumor. Additional to the examination imaging must be done. (7)

a) Sonography of the neck.

   This method allows the practitioner to quickly gain information about the lymph nodes regarding the enlarged nodes' count, size, and shape.

b) CT or MRI of the Neck.

   There should be images of the neck, the mediastinum, the pharynx, and the oral cavity. This way conclusions can be drawn about how big the extension of the tumor is. The tumor´s diameter and size are essential, even more its relations to the surrounding structures. Possible spreads to other parts of the pharynx, the larynx, the muscles of the tongue, the mandible, the skull base, or the carotid artery are thoroughly documented. These structures are essential because the TNM staging is partly based on infiltration of previous mentioned structures. (For a more detailed description see chapter 1.4.2.2) In addition, this imaging technique allows detecting metastatic spread to the lymph node or the soft tissue of the neck. The fact of how many and which lymph nodes are affected also play an important role in the staging of OPSCC.

c) Imaging of the thorax and the abdomen.

   To rule out distant metastases, additional imaging of thorax and abdomen is required. Usually, CT-scan is performed for the initial staging and an x-ray of the thorax and an ultrasound of the abdomen are sufficient for follow-up staging. However, if any suspicious lesion is detected, further diagnostic examinations must be done.

d) PET scan.

   A PET scan can be beneficial to identify a primary tumor, especially if the clinical examination did not lead to an adequate diagnosis of the primary tumor. PET scans can also determine additional distant metastases.

1.3.4 Obtaining tumor cells and histological examination

To diagnose an OPSCC properly, it must be confirmed by a pathologist. The histological workup of the cells includes a statement if it is malignant, the differentiation grade, and if it is related to HPV or not. There are different ways to obtain cells for analysis. Firstly, enlarged locoregional lymph nodes often are the first signal while the primary tumor can still be obscure. The initial diagnosis is based upon a cytology (fine needle aspiration method) or histology (core needle biopsy) sample taken from the lymph nodes, if they are visible and

easily accessible in the clinical examination, this is usually done with local anesthesia. The cooperation and compliance of the patient are required (3). Secondly, excisions of a single lymph node or parts of a lymph node can be taken under general anesthesia.

Unfortunately, the total extension of tumors cannot always be seen and accessed in the standard examination in an ambulant setting, sometimes a more thorough investigation during general anesthesia called panendoscopy is required.(7) A panendoscopy enables the surgeon to evaluate if the tumor is resectable or if its extensions favor a different therapy approach.

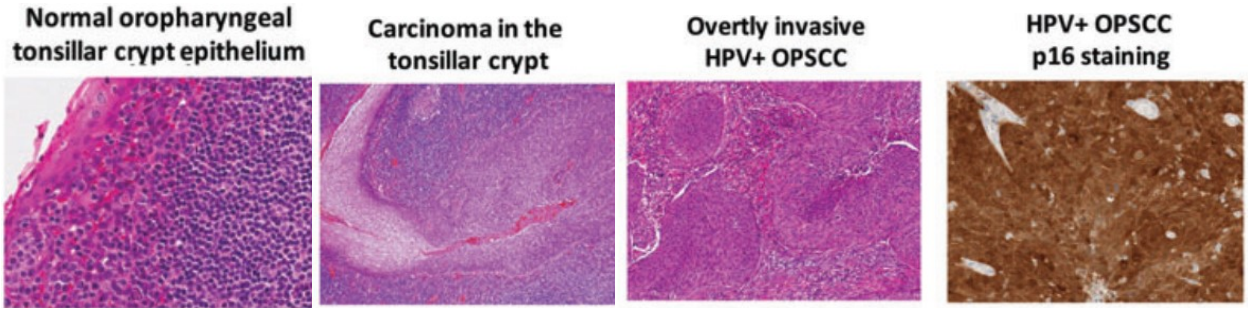
1.3.5 Evaluation of the HPV-status

It is of great importance to distinguish between HPV positive and negative carcinomas when it comes to OPSCC. There are different ways to detect this quality. The College of American Pathologists (14) state in their guidelines a recommendation that p16 immunohistochemistry (IHC) is the favoured technique for detecting HPV.

This widely used method is based on the surrogate marker p16 (INK4A), which means this technique does not detect HPV directly, but instead detects the reactions of the cells induced by a viral infection. During a transcriptionally active infection with high risk-HPV the E7 protein is overexpressed. It binds to the retinoblastoma protein (RB) and leads to the degradation of the tumor suppressor gene. If this happens, p16 protein is overexpressed, which can be detected with IHC. (15)

Definition of a p16-positive result is '*when there is at least 70% nuclear and cytoplasmic expression with at least moderate to strong intensity.'* ((14) p.564)

Figure 5 Histological depiction of p16+OPSCC



Courtesy of Kim et al., (15) p.214

'*The histological images represent normal oropharyngeal tonsillar crypt epithelium (×300, left), carcinoma in the tonsillar crypt (×200, second left), overtly invasive HPV+ OPSCC (×100, second right), and HPV + OPSCC p16 IHC staining (×200, right).*' p.214

Abbreviations: HPV: human papilloma virus, OPSCC: oropharyngeal carcinoma, IHC: immunohistochemistry

There are several reasons why this test is a good choice. Firstly, it is inexpensive and therefore affordable for public hospitals. Secondly, it is performed on FFPE, formalin-fixed, paraffin-embedded tissue, which can easily be realized with the samples already generated in the routine practice. No special preparation of the tissue needs to be done for this test (16). Thirdly, this test is highly sensitive, it detects transcriptionally active high-risk HPV in 94-100% (17, 18).

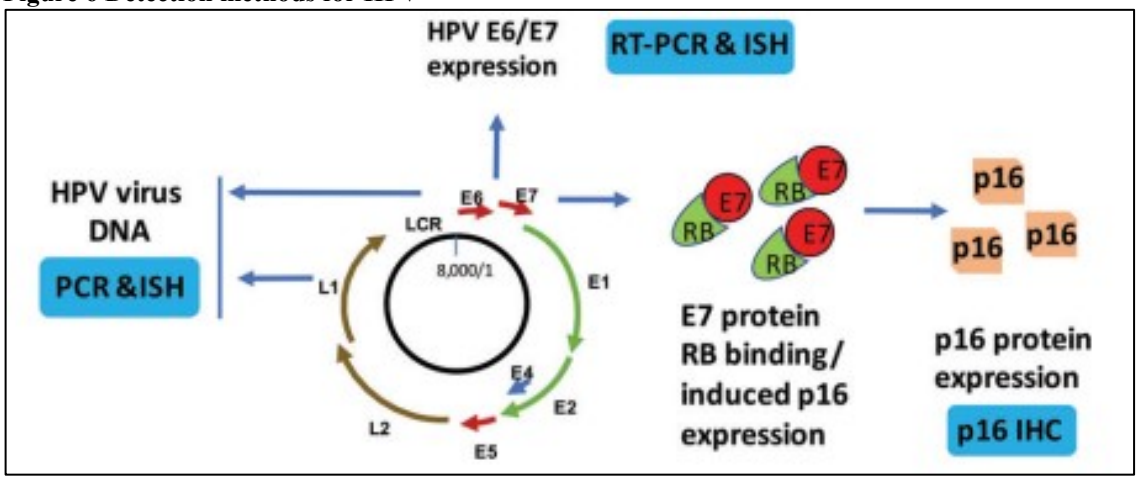
Although the sensitivity is very promising, also the specificity must be considered. According to Singhi et al.(18) and Schache et al. (17) specificity ranges from 79% to 82%. This low specificity can be explained because HPV-related tumors are not the only ones which overexpress p16. Even benign squamous cells or HNSCC unrelated to the human papilloma virus can have a positive result in the p16 IHC.(15)

Nauta et al. (19) criticize that these false positive results can cause problems. In their study tumor specimen underwent two tests for detecting HPV, p16 IHC and a test for HPV-DNA. Of all the patients classified as p16-positive, 12,4% had a negative result in HPV-DNA testing. For analyzing the outcome of the patients, they had three subgroups: patients with a p16pos/ HPV-DNA positive result, patients with a p16pos/ HPV-DNA negative result, and thirdly those patients who were p16-negative. Interestingly, the patients who were p16-positive, but HPV-DNA negative had a worse outcome than those with a p16-positive and HPV DNA result, their survival curve was similar to the one of the p16-negative subgroup. Therefore Nauta et al.(19) suggest that additional HPV DNA should be included in the OPSCC workup, especially if therapy-de-escalation is considered.

Other possible tests for detecting HPV are: (15)

a) Detection of HPV E6 & E7 mRNA using reverse transcriptase polymerase chain reaction (rt-PCR). This technique is often considered to be the gold-standard (15, 16)
a) Polymerase chain reaction (PCR) for detecting HPV DNA
b) In situ hybridization (ISH) for detecting HPV DNA
c) In situ hybridization (ISH) for detecting HPV RNA
d) Liquid-phase assays used for cytopathology.

Figure 6 Detection methods for HPV



Courtesy of Kim et al. (15) p.214
This figure shows the path-mechanisms and different detection methods of a human papillomavirus infection.
Abbreviations: DNA: desoxyribonucleic acid, HPV: human papilloma virus, PCR: polymerase chain reaction, RT-PCR: reverse transcription polymerase chain reaction, ISH: in situ hybridization, RB: Retinoblastoma protein, E6/E7: early genes 6 and 7, p16: p16 protein, IHC: immunohistochemistry

1.4 TNM Staging

1.4.1 Basics

For over 70 years cancers are being classified according to the TNM classification. It is undoubtedly advantageous as it helps the clinician plan treatments and predict a prognosis. As it is internationally used, information can be exchanged easily with other hospitals or doctors and in-between different specialties. This system is based on the disease's extension and every letter describes another anatomic region of the body that might or might not be affected.

T-Tumor: the extension of the tumor on the primary site

N-Nodes: absence or existence and the size of locoregional lymph node metastases

M- Metastasis: non-existence or presence and location of distant metastases

There are several additional descriptors too, but these are used for this thesis.

Moreover, there is a clinical, cTNM, and a pathological, pTNM, classification. The difference is how and when the information on the tumor is obtained. Before treatment, only a clinical classification can be realized. It is based on the evidence collected in variable examinations, such as physical exploration, imaging, or endoscopy. The pathological classification can be performed post-operatively, based upon the surgical specimen. (1)

1.4.2 TNM 8th Edition

The 8th Edition is the latest version of TNM cancer staging published. Even though it was released in January 2017, it took another year to implement it in clinical practice. Throughout 2017 (19) newly diagnosed cases should still have been classified with the 7th Edition, this year should guarantee that guidelines and protocols can be established. To sum it up, only patients newly diagnosed after January, 1st 2018 are classified with the latest version of TNM (20).

Two TNM classification manuals were published, one of the UICC, Union for Cancer Control (1), and one from the AJCC, American Joint Committee on Cancer (21). They correspond with each other, so there are no significant varieties. According to the UICC's manual, there are minor differences, but not concerning the chapter about the oropharynx (1).

1.4.2.1 Reasons for changes for the oropharynx

Several retrospective (22, 23) and prospective (24) studies, have proven that HPV positive tumors have a better prognosis than those caused by other risk factors. According to Ang et al. (23) the 3-year rates of overall survival of p16-positive OPSCC (*'83,6%, 95% CI, 78,7 to 88,6'* (23) p.30) significantly exceed the ones of the p16-negative subgroup, (*'51,3%, 95% CI, 41,5 to 61,0'* (23) p.30). Furthermore, also the disease-free survival showed better results (*'74,4 % 95% CI, 68,5 to 80,2'* (23) p.30) in p16-positive patients versus (*'38,4% (95% CI, 28,9 to 47,9'* (23) p.30) in p16-negative patients.

Not only the OS and the DFS are better in patients with HPV associated HNSCC, but they also respond better to treatment (24).

Another difference between HPV-positive and HPV-negative OPSCC is the presence of nodal metastasis at first presentation. HPV+OPSCC tend to have early nodal extension (25) and therefore present with an advanced nodal stage at initial examination (26). Due to this circumstances patients with HPV+ OPSCC are often staged as advanced disease following the rules of the 7th version of the TNM classification (27), still their prognosis is better than advanced-staged cases without association to HPV.

Due to these differences between HPV-positive and HPV-negative OPSCC, experts suggested that those two kinds of OPSCC should be treated as different entities. The TNM classification should reflect this separation. Based upon the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S) (28) the 7th version TNM staging has been modified. ICON-S conducted a retrospective multicenter cohort study with almost 2000 patients diagnosed with an HPV-positive OPSCC, including patients from seven different

cancer centers in Europe and North America. One of the institutions served as a training cohort and the other ones as a validation cohort.

1.4.2.2 Changes for the oropharynx

The suggestions of the ICON-S have been accepted and lead to an additional part about HPV-positive OPSCC included in the updated 8th TNM in 2017 (1).

To be precise, the 8th Edition of the TNM version addresses p16-positive OPSCC, not HPV-positive OPSCC. The distinction between the two forms of oropharyngeal cancers is solely based on p16 immunohistochemistry, other detection techniques are not applicable. p16-positivity is defined as an overexpression of p16 in IHC, all cases with a negative result and those where no p16 IHC was performed are classified as p16-negative. Modification concerns the p16-positive OPSCC, the classification of p16-negative OPSCC did not change, except for a minor amendment. (1)

1.4.2.2.1 Tumor extension: T category

As presented in the tables (Table 1, Table 2, Table 3) below, T categories remained almost the same. However, there is a difference for p16-positive OPSCC. The ICON-S proved in their survey that the 5-year overall survival of T4a and T4b diseases was almost equal (28). For this reason, T4b has been removed of the staging of p16-positive Thus, OPSCC, the two former subgroups are now classified as one T4-group (29).

Table 1 cT- 7th Edition UICC TNM Oropharynx

cT- 7th Edition UICC TNM Oropharynx	
T category	**T criteria**
T1	Tumor 2cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4a	Tumor invades any of the following: larynx *, deep/ extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate, or mandible
T4b	Tumor invades any of the following: lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base; or encases carotid artery

* Note: Mucosal extension to lingual surface of epiglottis from primary tumors of the base of the tongues and vallecula does not constitute invasion of larynx.

Sobin et al. (27) p.32

This table shows the categories of Tumor extension (T) as described in the UICC 7th Edition Tumor staging manual. (27)

Table 2 cT- 8th Edition UICC TNM Oropharynx p16-negative

cT- 8th Edition UICC TNM Oropharynx p16-negative-	
T category	**T criteria**
T1	Tumor 2cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4a	Tumor invades any of the following: larynx *, deep/ extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate, or mandible
T4b	Tumor invades any of the following: lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base; or encases carotid artery

* Note: Mucosal extension to lingual surface of epiglottis from primary tumors of the base of the tongues and vallecula does not constitute invasion of larynx.

Brierley et al. (1) p. 41

This table shows the Tumor extension categories (T) of p16-negative OPSCC as described in the UICC 8th Edition Tumor staging manual. (1)

Table 3 cT- 8th Edition UICC TNM Oropharynx p16-positive

cT- 8th Edition UICC TNM Oropharynx p16-positive	
T category	**T criteria**
T1	Tumor 2cm or less in greatest dimension
T2	Tumor more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumor more than 4 cm in greatest dimension or extension to lingual surface of epiglottis
T4	Tumor invades any of the following: larynx *, deep/ extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), medial pterygoid, hard palate, mandible, lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, skull base; or encases carotid artery

* Note: Mucosal extension to lingual surface of epiglottis from primary tumors of the base of the tongues and vallecula does not constitute invasion of larynx.

Brierley et al. (1) p.42

This table shows the clinical extension of the tumor (cT) category for p16-positive OPSCC as described in the UICC 8th Edition Tumor staging manual. (1)

1.4.2.2.2 Nodal extension: N category

The N-category was without a doubt the one with the most modifications, especially for p16-positive OPSCC, as seen in Table 6 below. The results of the ICON-S study showed that the 5-year overall survival of p16-positive patients with N1 (1 unilateral lymph node < 3cm), N2a (1 unilateral lymph node > 3cm) or N2b (>1 unilateral lymph nodes) is almost identical. (28) In other words, as long as the involvement of the lymph nodes is restricted to one side of the neck and smaller than 6 cm, the prognostic impact is the same. That is why these three former subgroups have been summed up to one category, N1. Lymph nodes are classified as N2 if there is a bilateral or contralateral involvement. A size bigger than 6 cm, no matter if ipsi- or contralateral, predicts a worse outcome and therefore these nodes are classified as N3 in HPV positive OPSCC. (29)

In addition, also the N-Classification of p16-negative OPSCC (Table 5) was slightly modified, stage N3b was added compared to the 7th version (27) (Table 4). Lymph nodes are classified this way if there is profound evidence of extra nodal extension (ENE), which implies the worst outcome of regional metastatic spread. To diagnose ENE, radiologic proof is not enough, there must also be clinical evidence such as invasion of the skin or dysfunction of a nerve, e.g., the sympathetic trunk. (29)

Table 4 cN- 7th Edition UICC TNM Oropharynx

cN- 7th Edition UICC TNM Oropharynx		
N category		**N criteria**
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6cm in greatest dimension
	b	Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension
	c	Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension
N3		Metastasis in a lymph node more than 6 cm in greatest dimension

Note: Midline nodes are considered ipsilateral nodes.

Sobin et.al(27) p.34

This table shows the categories of lymph nodal extension (N) as described in the UICC 7th Edition Tumor staging manual (27).

Table 5 cN- 8th Edition UICC TNM Oropharynx p16-negative

cN- 8th Edition UICC TNM Oropharynx p16-negative		
N category		**N criteria**
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6cm in greatest dimension
	b	Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension
	c	Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension
N3	a	Metastasis in a lymph node more than 6 cm in greatest dimension without extra nodal extension
	b	Metastasis in a single or multiple lymph nodes with clinical extra nodal extension *

Notes: *The presence of skin involvement or soft tissue invasion with deep fixation/tethering to underlying muscle or adjacent structures or clinical signs of nerve involvement is classified as clinical extra nodal extension.

Midline nodes are considered ipsilateral nodes.

Brierley et al. (1) p.43

This table shows the categories of lymph nodal extension (N) for p16-negative OPSCC as described in the UICC 8th Edition Tumor staging manual.(1)

Table 6 cN- 8th Edition UICC TNM Oropharynx p16-positive

cN- 8th Edition UICC TNM Oropharynx p16-positive	
N category	**N criteria**
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Unilateral metastasis, in lymph node(s), all 6cm or less in greatest dimension
N2	Contralateral or bilateral metastasis in lymph node(s), all 6 cm or less in greatest dimension
N3	Metastasis in lymph node(s) greater than 6 cm in dimension

Note: Midline nodes are considered ipsilateral nodes.

Brierley et al. (1) p.43

This table shows the categories of lymph nodal extension (N) for p16-positive OPSCC as described in the UICC 8th Edition Tumor staging manual. (1)

1.4.2.2.3 Distant metastasis: M-category

The category of distant metastasis (M) remained the same in the 8th version of TNM (1, 27). Thus, M0 means there are no distant metastases and M1 there is a distant metastasis.

1.4.2.2.4 Stage groups

The tables below clearly depict how the stage groups differ between p16-positive (Table 9) and p16-negative OPSCC (Table 8). Patients with p16-negative OPSCC only belong to stage I and II if there are no nodal metastases, everything more than N1 immediately equals stage III. In contrast, patients suffering from p16-positive OPSCC are in stage III with N3, meaning lymph node metastases bigger than 6 cm.

Another difference is the predictive stage group IV, in the p16-positive subgroup patients are only classified as stage IV if there are distant metastases whereas in the p16-negative group stage IV is divided in 3 subgroups and patients are classified as stage IVA, IVB or IVC.

The conclusion drawn from this tables (Table 8,Table 9) is that patients classified with advanced disease stage (III and IV), present with bigger sized tumors and more locoregional metastasis in p16-positive OPSCC group.

Table 7 7th Edition UICC TNM Oropharynx – Stage grouping (clinical)

7th Edition UICC TNM Oropharynx – Stage grouping (clinical)			
Stage	**Stage criteria**		
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IVA	T1, T2, T3	N2	M0
	T4a	N0, N1, N2	M0
Stage IVB	T4b	Any N	M0
	Any T	N3	M0
Stage IVC	Any T	Any N	M1

Sobin et al. (27) p.36

This table shows the prognostic stage groups as described in the UICC 7th Edition Tumor staging manual.(27)

Table 8 8th Edition UICC TNM Oropharynx p16-negative –Stage grouping (clinical)

8th Edition UICC TNM Oropharynx p16-negative – Stage grouping (clinical)			
Stage	**Stage criteria**		
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IVA	T1, T2, T3	N2	M0
	T4a	N0, N1, N2	M0
Stage IVB	T4b	Any N	M0
	Any T	N3	M0
Stage IVC	Any T	Any N	M1

Brierley et al. (1) p.45

This table shows the prognostic stage groups of p16-negative OPSCC as described in the UICC 8th Edition Tumor staging manual. (1)

Table 9 8th Edition UICC TNM Oropharynx p16-positive –Stage grouping (clinical)

8th Edition UICC TNM Oropharynx p16-positive –Stage grouping (clinical)			
Stage	**Stage criteria**		
Stage 0	Tis	N0	M0
Stage I	T1, T2	N0, N1	M0
Stage II	T1, T2	N2	M0
	T3	N0, N1, N2	M0
Stage III	T1, T2, T3	N3	M0
	T4	Any	M0
Stage IV	Any T	Any N	M1

Brierley et al. (1) p.45

This table shows the prognostic stage groups of p16-positive OPSCC as described in the UICC 8th Edition Tumor staging manual. (1)

1.5 Therapy

1.5.1 Multidisciplinary decision-making

To obtain the best outcome for the patient, it is recommended that patients with OPSCC should be treated in multidisciplinary team, consisting of Head-and-neck-surgeons, oncologists, radiologists, pathologists, specialized nursing care, members of rehabilitation care, etc. The base of decision making is the TNM staging system, but additional information must be taken into consideration (30). Relevant details are for example the age of the patient, comorbidities and the ECOG performance score. The ECOG score is a commonly used tool to evaluate how much the disease restrains patients in their daily lives. Moreover, it is advantageous during therapy, as it can easily indicate a decrease of the patient's condition. (31)

Table 10 ECOG Performance Status

ECOG Performance Status

Developed by the Eastern Cooperative Oncology Group, Robert L. Comis, MD, Group Chair.*

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol.* 1982;5:649-655.

Oken et al. as cited by ECOG-ACRIN Cancer Research Group (31)

This table shows the ECOG score which stands for Eastern Cooperative Oncology Group. This score has six grades and helps physicians worldwide to evaluate the physical performance of patients with cancer.

Furthermore, as some therapy regimens require patients collaborating with their doctors, so the compliance of the patient is of utmost importance. Finally, as patients cannot be forced to have a therapy, the patient's wishes and consent affect the decision making strongly.

1.5.2 Curative treatment

A curative intent means that the patient can be potentially cured. There are different ways to obtain the goal.

1.5.2.1 Surgery

To begin with, if feasible a surgical approach should be tried. The goal is to eliminate the whole tumor as remaining tumor cells indicate a worse prognosis. Usually an OPSCC-surgery consist of two parts. Firstly, the resection of the tumor on the primary site.

Possible options for tumor removal are: (3)

1. Transoral. A typical indication for this approach is a T1/T2 tumor of the palatine tonsil. In this case, a tonsillectomy can be performed (3).
2. Laser resection. This method is typically used for small tumors of the base of the tongue (3).
3. Open approach and reconstruction. Advanced tumors can be in the need of an extensive surgery. If larger parts of the pharynx are removed, the defect must be covered, usually a radialis flap is used (3).

Secondly, in addition to the tumor resection a neck dissection can or cannot be conducted. There a three different forms of neck dissections. (3)

1. Radical Neck Dissection. Level I to V, the sternocleidomastoid muscle, the internal jugular vein and the accessory nerve are removed.
2. Modified Radical Neck Dissection. In comparison to the radical neck dissection, solely the Levels I to V are removed, the other mentioned structures can be retained if there is no sign or evidence of tumor invasion.
3. Selective Neck Dissection. As mentioned before HNSCC tend to metastasize only to specific Levels, so depending on the primary site of the tumor only these Levels are removed, the others can be preserved. This procedure should only be performed if there is no clinical evidence of locoregional metastases, thus only N0. In the case of oropharyngeal cancers, the Levels II to IV are removed.

Neck dissections can be performed unilateral or bilateral depending on the involvement of the lymph nodes.

If it becomes apparent in the finally histology report that there are residual tumor cells, another surgery can be performed or otherwise adjuvant radiotherapy/ chemoradiotherapy is recommended (30). In addition, intraoperative frozen section analysis is performed regularly to prevent residual tumor lesions because examination results will have immediate consequences on the subsequent surgical procedure.

Surgery should not be favored, if the surgery would cause a significant impairment of the functions for the patient or if the extension involves critical structures, thus in advance total extraction seams unfeasible. In addition, as HPV-positive OPSCC respond better to chemoradiotherapy than HPV-negative OPSCC, a primary conservative approach should be considered (3). However, this opinion is discussed controversy in the current literature. According to Ott et al. (32) there is a lack of convincing results of prospective randomized trials whether a primary radiotherapy or a primary surgery should be preferred. De-escalation

in tumor therapy in patients with HPV positive OPSCC should be considered thoroughly, not only because of under-treatment but also because not all p16-positive OPSCCs are 100% HPV driven carcinomas.

1.5.2.2 Radiotherapy

Radiotherapy, in combination with chemotherapy, is another option for primary OPSCC therapy. One complete treatment cycle lasts typically seven weeks, the patients have their sessions every workday. Usually 70 Gray (35 x 2 Gray) are applied at the primary tumor site. If there is no clinical evidence of metastatic spread, the region of the lymph nodes can be irradiated with a lower dosage, to reduce toxicity. A contrast-enhanced tumor imaging is fundamental for radiotherapy to plan the exact area that needs to be irradiated. In addition, there are techniques like IMRT (Intensity-Modulated Radiation Therapy) that only affect the tumor and the surrounding structures are mostly spared, again to reduce radiotoxicity-related events.

Radiotherapy can be conducted alone in early-stage diseases (T1-T2, cN0/ single node <3cm) without ENE or with a concurrent systemic therapy in advanced disease (T3-T4, N2-3) with ENE. (30)

1.5.2.3 Systemic therapy

For a curative intent it is not recommended to use systematic therapy alone without another form of treatment. For example, it can be applied with concurrent radiotherapy. The preferred antineoplastic systematic agent for this purpose is high dose Cisplatin (100mg/m^2 body surface area). Another commonly used agent is Cetuximab. However, in 2019 the results of a prospective randomized trial were published in the Lancet by Gillison et al. (33), which indicate that the performance of Cetuximab is inferior to Cisplatin.

Even though it obtains the best results, not all patients can receive a treatment with Cisplatin. This antineoplastic agent has a high rate of acute toxicity. Therefore, patients with certain pre-existing conditions, such as renal impairment or deafness, are not eligible for a Cisplatin-based therapy. In these cases, patients must be treated with a different systemic therapy, carboplatin as well as cetuximab can be used as a substitute (34).

Secondly, adjuvant chemotherapy can be suggested in advanced disease. For this approach, a combination of docetaxel/cisplatin/5-fluorouracil is commonly used. This regimen is then followed by a concurrent chemo-radiotherapy as described above (30). However, it should be noted that there are some concerns regarding the high toxicity of platinum-based induction-chemotherapy followed by a concurrent therapy with Cisplatin (35).

1.5.3 Palliative treatment and best supportive care

Not every tumor is curable, but that does not mean that patients get no treatment. It just means that the aim of the treatment changes, not the total curation of the patients, is the highest goal but rather maximizing the patient's quality of life. Two approaches are sharing this goal, called palliative therapy and best supportive care (BSC). In published literature these two terms are often hard to be distinguished as they overlap very often but they still have significant differences (36). According to Jassem et.al (37) best supportive care is '*treatment administered with the intent to maximize quality of life without a specific antineoplastic regimen.*' p. 1699. So, one can say that the main difference is that palliative treatment still includes tumor specific therapy whereas BSC does not. Patients treated with a palliative intent still receive chemotherapy, immunotherapy, palliative surgeries, or palliative radiation, but these procedures should primarily reduce symptoms. For example, patients having swallowing problems can undergo a palliative radiation or the mass can be reduced surgically. Another example are metastases to the bone, these usually get irradiated to relieve pain or to prevent paralysis (38). But all the previous described therapies still have their risks and toxicities; therefore, they can only be applied to patients with a decent health condition. Patients with poor performance status cannot endure these procedures and thus can only obtain best supportive care (30). The main goals are securing nutrition and breathing, reducing pain and therapy induced problems like nausea, constipation etc. (38).

1.5.4 Follow up care

It is strongly recommended that patients who have survived an oropharyngeal neoplasm regularly attend a clinician for a follow up examination. Patients treated at Medical University of Graz, Department of Otorhinolaryngology are summoned every 3 months during the first two years and every 6 months during the 3rd, 4^{th,} and 5th year after treatment. After 5 years of no progression of the initial OPSCC, patients are advised to visit an ENT physician in private practice once a year.

A follow up examination should consist of a thorough head and neck examination, also mirror and fiberoptic exams should be conducted. Furthermore, according to the NCCN guidelines (30), annual reimaging of areas that are impossible to see during clinical examination is indicated. Therefore, patients of the Department of Otorhinolaryngology at the Medical University of Graz must bring radiological imaging of their neck, thorax, and abdomen once a year.

These sessions aim to detect recurrences fast, allowing quick therapy and enhancing the patient's prognosis.

1.6 Lack of knowledge

As mentioned above, it is established knowledge in published literature today, that the HPV status of an OPSCC has a great influence on the outcome of patients. The current 8th Edition of the UICC TNM staging manual (1) reflects the difference of p16-positive and p16-negative OPSCC. There are several studies published around the world evaluating its validity and many authors (25, 39, 40) claim that the 8th Edition of UICC TNM classification (1) is an improvement, however the authors note a lack of clarity in the distinction between some stages. Further studies must be conducted to determine which aspect of the TNM staging manual needs refinement or other ways to improve the validity of prognoses. Another question not sufficiently answered yet is if the p16 status should affect therapy decisions concerning OPSCC. Ongoing research might determine whether p16-positive OPSCC should be treated differently, respectively if lesser doses of antineoplastic agents are sufficient (41).

1.7 Purpose of this study

The main aim of this study was to see if the data of a tertiary care center in Styria can also support the benefit of the application of the new 8th Edition of UICC TNM classification (1) compared to its predecessor. Moreover, it should shed some light on other questions of interest, e.g., potential aspects of the TNM system which could be improved and the influence of p16-status on therapy outcome. Additionally, the study should summarize general information about patients with OPSCC in Styria and evaluate the adaption to the new staging system.

1.7.1 Main hypothesis

The main hypothesis of this thesis is the following: The new 8th Edition of UICC TNM classification shows more significant differences in OS/DFS between the individual stages (I-IVC) regarding patients with OPSCC.

Null hypothesis for statistical calculations:

- H0: There is no significant difference between the individual stages in OS / DFS regarding p16-negative/-positive OPSCC applying the 7th/ 8th Edition of TNM classification.

1.7.2 Side hypothesis

The first side hypothesis is that T respectively N categories as established in the 8th Edition of UICC TNM classification show more significant differences in OS/DFS between the individual T categories (1-4b) respectively N categories (N0-N3b) regarding patients with OPSCC. The second side hypothesis is that p16-status leads to different therapy strategies.

2 Material and methods

2.1 Patients

Most patients were referred to Medical University of Graz, Department of Otorhinolaryngology from an ENT specialist in private practice with a suspected oropharyngeal neoplasm or a suspect swelling of a lymph node to further investigate the disease. As the Medical University of Graz, Department of Otorhinolaryngology is a tertiary medical care center, some patients with advanced disease were referred from a peripheral hospital in Styria or southern Burgenland for treatment decisions and execution. We have observed that patients, especially from south Styria, present with high tumor stages at first clinical presentation.

2.1.1 Inclusion criteria

Patients were eligible for our analyses if:

- they suffer from an oropharyngeal squamous cell carcinoma,
- were initially diagnosed in the period of 01.01.2015 to 31.12.2018,
- are primarily treated at the Medical University of Graz, Department of Otorhinolaryngology

2.1.2 Exclusion criteria

During our research, some patients proved themselves not eligible for our analyses. We had to exclude patients without a histological confirmation of their disease. It turned out that some patients have a history of HNSCC before 2015. Those patients were not initially diagnosed in this defined period, instead they were either presented because of a secondary carcinoma, a residual tumor, or a remission of an earlier HNSCC and therefore cannot be considered in our analyses. Some patients only got a secondary opinion and were treated elsewhere, they also were excluded. Finally, some patients had another histological entity instead of a squamous cell carcinoma, for example a B-cell lymphoma of the palatine tonsil mistook for an SCC. Those patients were also not eligible.

2.1.3 Informed consent

Approval was obtained from the Ethic Board of Medical University of Graz prior to performance of the study which involved waiver of consent for individual patients. Of course, all personal data was treated with respect and was pseudonymized.

2.2 Objectives

2.2.1 Main objectives

As one aim of this work is to prove, that it matters whether an oropharyngeal carcinoma is associated with HPV or not regarding the outcome for the patient, overall survival (OS) und disease-free survival (DFS) were the main endpoints that were analyzed. In addition, especially the differences between the two groups of patients, p16-positive group and p16-negative group were of interest.

Furthermore, this work's main focus is set on the latest version of TNM classification (UICC 8th Edition (1)), if the separation between p16-positive and p16-negative OPSCC patients and adapted T and N classifications allow to give a better prognosis. Thus, several survival functions were calculated, applying both the 7th Edition (27) and the 8th Edition (1), which then could be compared. Again, OS and DFS were the main objectives for this evaluation.

Overall survival was defined as the period beginning with the date of diagnosis until death by any cause, respectively disease-free survival as the period from the date of diagnosis to the date of diagnosis of any disease progression.

2.2.2 Secondary objectives

In addition, this work shed some light upon the situation of patients suffering of oropharyngeal cancers in Styria, therefore we also analyzed information about the ECOG at initial presentation, differences between gender, applied therapy and how the chosen therapy strategy influenced the OS and DFS of the patients. In addition, we evaluated if the separate analysis of T and N stage individually allows to give a better prognosis for patients. Furthermore, we wanted to evaluate the adjustment to the 8th UICC TNM classification (1) in the Head and Neck Tumor Board.

2.3 Methods

2.3.1 Data collection

The data for our analysis was compiled throughout a year. To gain all the information about the patients, medical records were read, and necessary details were extracted. This information was comprehensible and password-saved documented in the REDCap program (42).

The following reports were of particular interest for our analyses:

1. Head and Neck Tumor Board. Records of the Head and Neck Tumor Board report and the decisions concerning the therapy. Special attention was drawn to the TNM

classification, which was adapted by the interdisciplinary team of the Head and Neck Tumor Board.

2. Pathohistological reports. Histological criteria documented via the record of the pathologist. First and foremost, the p16-status was of interest, but also the grading of the tumor. Unfortunately, the p16 status could not be evaluated in all patients, some needed to be noted as “no info”.
3. Radiological reports. Records of the radiologist are of great importance for the staging of the tumor; Not only the extent of the primary tumor but also possibly spreads to lymph nodes and distant metastasis have an impact on the tumor classification. Unfortunately, the radiological staging was incomplete in some patients, this was noted.
4. Operation report. Details about the surgery were collected.
5. Report letter (outpatient and inpatient clinic). Data about details and applied dosages of conservative therapies were collected, e.g., chemotherapy, immunotherapy, and radiotherapy.
6. Follow up. The medical records were searched for progress of the disease, such as second malignomas, local recurrence, distant metastasis, or death. The follow up of all patients was last checked in April 2020.

Of secondary importance for the analyses was information about comorbidities and past/present alcohol intake and nicotine consumption, those details were also documented for completeness.

2.3.2 Tumor classification

For our analyses, each patient received three TNM classifications. Firstly the official one from the Head and Neck tumor board, secondly all patients were-in synopsis with all medical records- restaged by two research associates according to the 7th Edition (27) and thirdly according to the 8th Edition of UICC TNM classification (1).

2.3.3 Statistical analysis

As appropriate, continuous data are described as mean and standard deviation or median and range (minimum to maximum). Categorical parameters are summarized as absolute and relative frequencies. Differences between the p16-positive and p16-negative groups were assessed using Student's t, the Mann–Whitney U, or Fisher’s exact test. In addition, disease-free and overall survival were analyzed, in the whole cohort as well as separately for p16-negative and p16-positive patients, by Kaplan-Meier curves and the log-rank test was used

to test for group differences. This also applies for the validation of the 8th UICC TNM classification (1), for this analysis differences in OS respectively DFS between the individual UICC stages were calculated.

Statistical analysis was performed with SPSS statistical software, version 25.0 (IBM SPSS Statistics, Chicago, IL) and R version 3.6.1. P values <0.05 are considered statistically significant.

2.3.3.1 Considerations

Of great importance for our analysis is the p16 status, as many calculations compare p16-positive and p16-negative OPSCC. However, in 11 cases the p16- status could not be evaluated. Therefore, it should be noted that the patient with the uncertain p16-status occupy a special position in our analysis. In every calculation that differs, specifically between p16-positive and p16-negative patients, those patients with unknown p16-status were not taken into account, to prevent bias in result. However, in all the evaluation of the whole study group where p16 status does not matter they are included.

In four cases, the detailed tumor extent was not documented in the initial Head and Neck Tumor Board, neither was this information to be found retrospectively. Therefore, these four patients were excluded when the tumor extent was evaluated separately.

Moreover, it should be noted that follow up duration varies among our study population. As just a few even had the possibility of being followed up longer than 3 or 4 years we decided only to analyze 42 months of following the patients, minimizing falsification of our data for said period.

3 Results

A total of 178 patients could be included in our analyzes. Of these 178 patients 20,8% (n=37) were female, the vast majority with 79,2% (n=141) was male. The mean age at diagnosis of the whole group was 64,16 years. The tumor was located in the tonsils in 43,8% (n=78), in the base of the tongue in 28,7% (n=51), in the palatal arch in 3,9% (n=7), in other sites of the oropharynx in 20,2% (n=36), and on multiple sites in 3,4% (n=6).

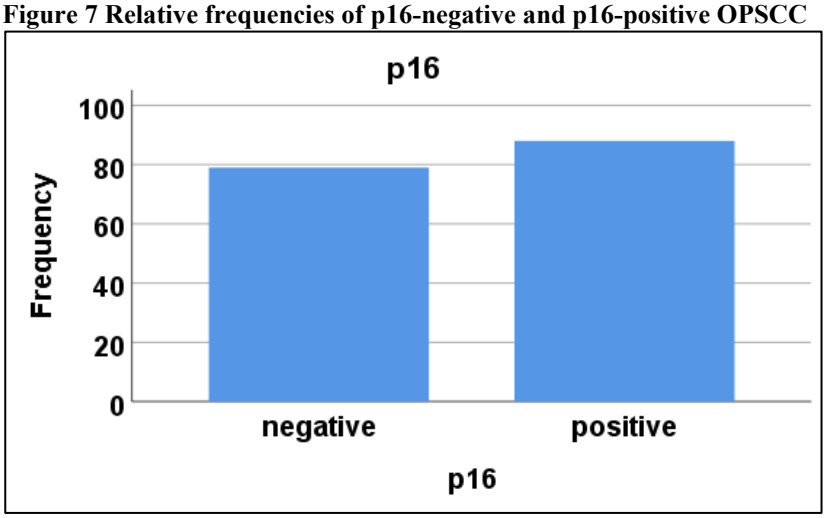
For our analyzes patients were divided into several groups.

To start with, we formed subgroups depending on p16- status. In our study population 44,4 % (n=79) tumors were classified as p16-negative, 49,4% (n=88) were p16-positive and in 6,2% (n=11) of the cases p16 status could not be determined in the medical records.

Moreover, patients were grouped according to the advance of their disease. Tumors were classified as early-stage (stage I and II) or advanced-stage disease (stage III and IV). Considering all patients of our study group, 39,9% (n=71) were classified as early-stage disease and 59,0% (n=105) as advanced-stage disease. In 1,1% (n=2) this information could not be assessed retrospectively.

Furthermore, patients were pooled in subgroups depending on whether they were classified before (< 2018) or after 2018 (≥ 2018). There were 139 patients in the subgroup before 2018 and 39 patients from 2018.

Figure 7 Relative frequencies of p16-negative and p16-positive OPSCC



This figure shows the relative frequencies of p16-negative and p16-positive patients.

3.1 Tumor

3.1.1 Localization

We took a closer look on the distribution of the tumor in the oropharynx and on the tumor side to find out if p16-positive OPSCC differed in their appearance compared to the p16-negative ones.

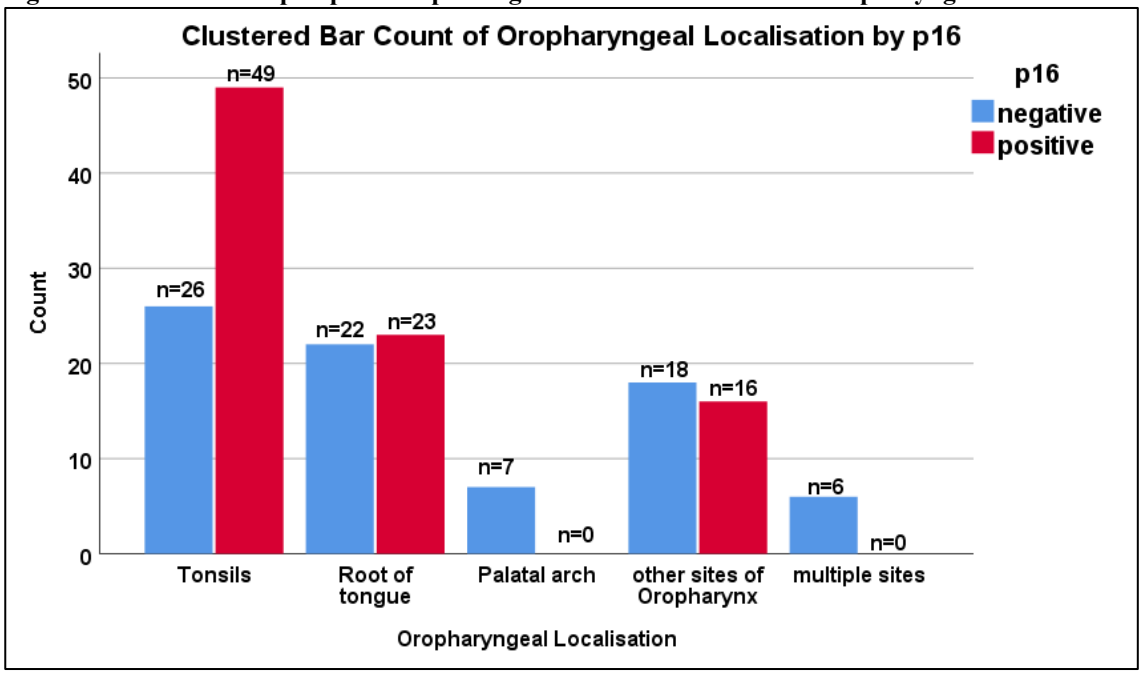
3.1.1.1 p16-negative OPSCC

32,9% (n=26) of the p16-neagtive tumors were located in the tonsils, 27,8% (n=22) in the base of the tongue, 22,8% (n=18) were located in other sites of the oropharynx, 8,9% (n=7) were found in the palatal arch and 7,6% (n=6) presented with tumor tissue in multiple sites, as outlined in Figure 8. In this group 39,2% (n=31) tumors were located on the right side, 38,0 % (n=30) on the left side and 13,9 (n=11) were crossing the center line. In 7 patients (8,9%) the tumor side was not documented in the first examination.

3.1.1.2 p16-positive OPSCC

55,7% (n=49) of the p16-positive tumors were located in the tonsils, 26,1% (n=23) in the base of the tongue and 18,2% (n=16) were located in other sites of the oropharynx, as outlined in Figure 8. In this group 44,3% (n=39) tumors were located on the right side, 42,0 % (n=37) on the left side and 5,7% (n=5) were crossing the center line. In 7 patients the tumor side was not documented in the first examination.

Figure 8 Distribution of p16-positive/ p16-negative OPSCC on different oropharyngeal locations



This clustered bar chart depicts the absolute frequencies of p16-negative OPSCC (blue) and p16-positive OPSCC (red) on the different oropharyngeal locations.

3.2 Patients' traits

3.2.1 Gender

Of all 178 included patients 20,8% (n=37) were female, the vast majority with 79,2% (n=141) was male. As outlined in Table 11, in the p16-positive subgroup consisting of 88 patients, 25,0% (n=22) were female and 75,0% (n=66) were male. In the p16-negative subgroup consisting of 79 patients, 17,7% (n=14) were female and 82,3% (n=65) were male.

This analysis revealed that most of the patients with oropharyngeal carcinomas treated at Medical University of Graz, Department of Otorhinolaryngology were men. Women were only a small part of the patient collective. In the male group patients were almost equally divided between the p16-positive (50,4%, n=66) and the p16-negative subgroup (49,6%, n=65). In contrast, 61,1% (n=22) of women were p16-positive and 38,9% (n=14) were p16-negative. However, this difference was not statistically significant (Pearson Chi Square, p=0,253).

Table 11 Crosstabulation p16-status* Gender

Gender * p16 Crosstabulation					
			p16		
			negative	positive	Total
Gender	female	Count	14	22	36
		% within Gender	38,9%	61,1%	100,0%
		% within p16	17,7%	25,0%	21,6%
		% of Total	8,4%	13,2%	21,6%
	male	Count	65	66	131
		% within Gender	49,6%	50,4%	100,0%
		% within p16	82,3%	75,0%	78,4%
		% of Total	38,9%	39,5%	78,4%
Total		Count	79	88	167
		% within Gender	47,3%	52,7%	100,0%
		% within p16	100,0%	100,0%	100,0%
		% of Total	47,3%	52,7%	100,0%

This crosstabulation shows the distribution of p16 prevalence among gender.

3.2.2 Age at time of diagnosis

In published literature, it is common understanding that p16-positive OPSCC appear earlier in patients than their p16-negative counterparts. (Mehanna et al. as cited by (29))

As outlined in Table 12, the mean age at diagnosis in the p16-negative subgroup (62,10±10,2years) in our study group was lower than in the p16-positive subgroup (66,11±9,7 years). In other words, in our study cohort, patients with p16-negative OPSCC were younger when diagnosed with their disease than those with a p16-positive OPSCC, this difference was statistically significant (p=0,010).

Table 12 Comparison of age at diagnosis by p16 status

Group Statistics					
	p16	N	Mean	Std. Deviation	Std. Error Mean
Age at first diagnosis	negative	79	62,10	10,239	1,152
	positive	88	66,11	9,683	1,032

This table depicts the means of age at diagnosis compared by p16 positivity/negativity.

In addition, we analyzed if there was also a difference in the age of diagnosis between men and women. Even though the female average, 65,4 years, was a little higher than the male one, 63,8 years, there was no statistically significant difference between the age of diagnosis in female or male patients (p=0,388).

3.2.3 ECOG

Patients with p16-positive OPSCC are not only known to be younger but also healthier. One way to describe their healthiness and ability to cope with their daily life is the ECOG performance score.

3.2.3.1 p16-negative OPSCC

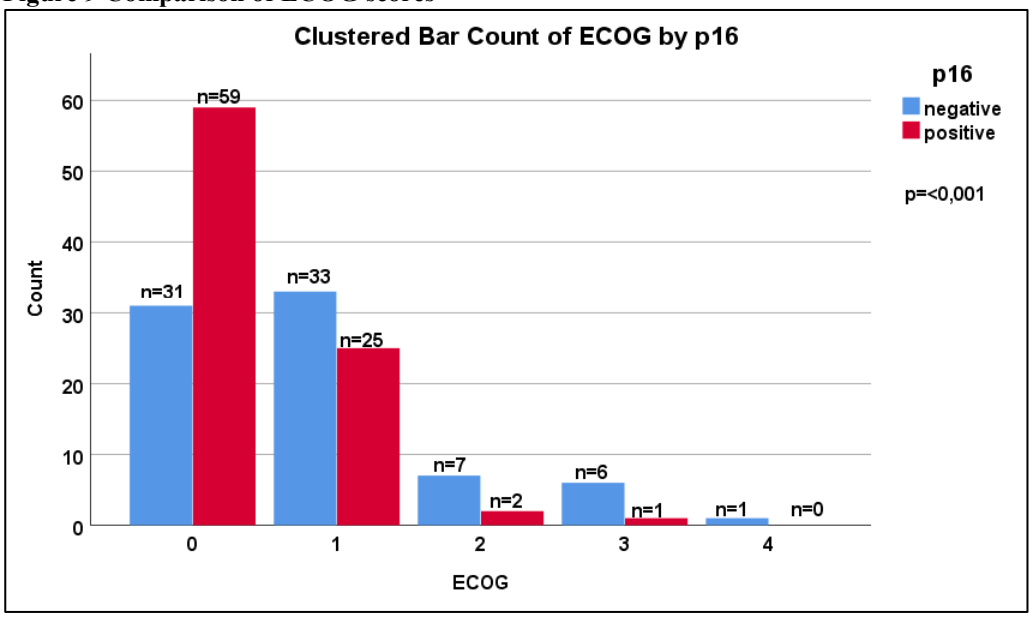
In the p16-negative subgroup consisting of 79 patients, 39,2% (n=31) scored an ECOG 0, 41,8% (n=33) an ECOG 1, 8,9% (n=7) patients an ECOG 2, 7,6% (n=6) an ECOG 3 and 1,3 (n=1) an ECOG 4. In one patient (1,3%) there was no ECOG score available.

3.2.3.2 p16-positive OPSCC

In the p16-positive subgroup consisting of 88 patients, 67,0% (n=59) scored an ECOG 0, 28,4% (n=25) an ECOG 1, 2,3% (n=2) patients an ECOG 2, 1,1% (n=1) an ECOG 3. Of one patient (1,1%) there was no ECOG score available.

Comparing the patients of the p16-negative and p16-positive subgroups showed a difference in the performance of the patients at initial presentation, as seen in Figure 9. This difference was statistically significant (Mann-Whitney U Test p<0,001).

Figure 9 Comparison of ECOG scores



This clustered bar chart depicts the distribution of ECOG scores of patients with p16-negative (blue) and p16-positive (red) OPSCC. Abbreviations: ECOG= Eastern Cooperative Oncology Group, stands for a tool used to evaluate the performance of patients with cancer.

3.3 Outcome

3.3.1 Overall survival

One of the main topics of this work was to analyze the difference in survival between the patients suffering from a p16-positive OPSCC compared to the p16-negative ones. The status of life or death was last checked in April 2020. Therefore, we analyzed 42 months of follow up.

3.3.1.1 Whole study population

In the whole study group (n=178) 135 patients (75,8 %) were alive and 43 patients (24,2%) already deceased at time of data collection.

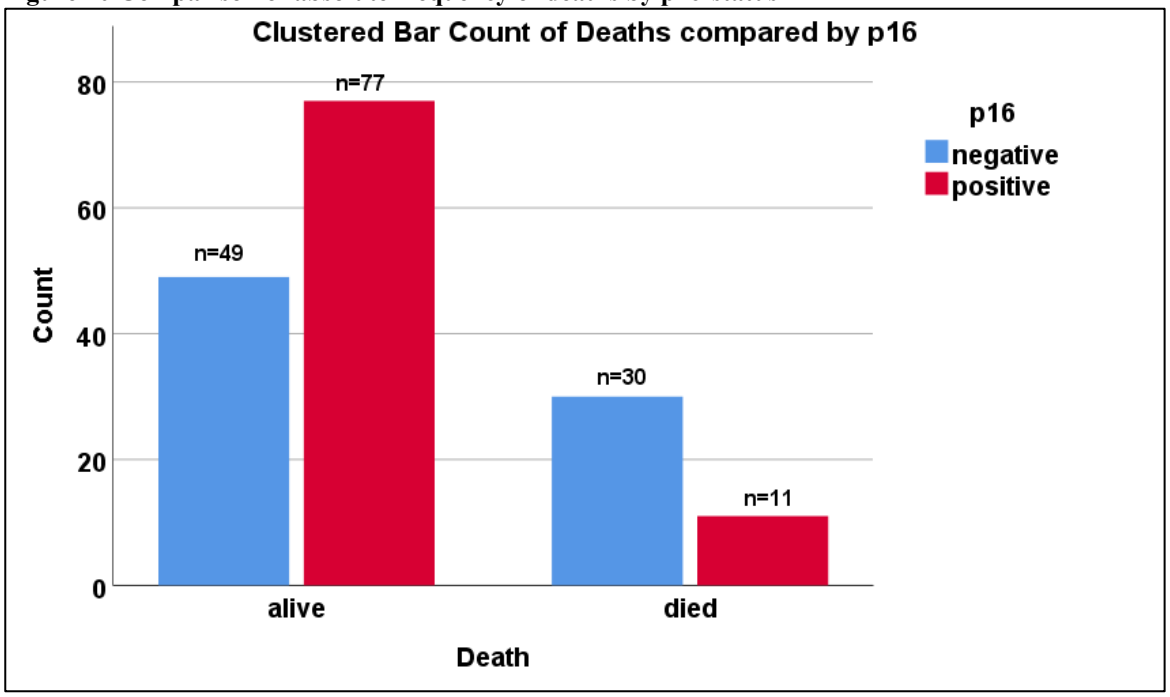
3.3.1.2 p16-negative OPSCC

In the p16-negative group (n=79) 49 patients (62,0%) were alive at the time of data collection and 30 patients (38,0%) were already deceased, as outlined in Figure 10.

3.3.1.3 p16-positive OPSCC

In the p16-positive group (n=88) 77 patients (87,5%) were alive at the time of data collection and 11 patients (12,5%) already deceased, as outlined in Figure 10.

Figure 10 Comparison of absolute frequency of deaths by p16-status



This clustered bar chart depicts death in patients with p-16 negative (left) and p16-positive (right) OPSCC.

3.3.1.4 Overall survival functions

Analyzing all OPSCC of our study group together, the 1-year OS rate was 84,8% and the 3-year OS-rate was 71,8%, the mean OS was 33,9 months (95% CI: 31,7-36,0).

For p16-positive OPSCC the 1-year OS-rate was 95,3% and the 3-year OS-rate was 84,6%, the mean OS was 38,3 months (95% CI: 36,2-40,4).

For p16-negative OPSCC the 1-year OS-rate was 71,9% and the 3-year OS-rate was 54,4%, the mean OS was 28,1 months (95% CI: 24,2-32,1).

Patients with a p16-negative OPSCC had a worse overall survival than those with a p16-positive OPSCC, this difference was statistically significant (p<0,001).

This proves that the distinction between the two groups is beneficial for the sake of venturing a prognosis, as outlined in Figure 11a and b.

Figure 11 OS a) whole study group b) comparing p16-positive to p16-negative OPSCC

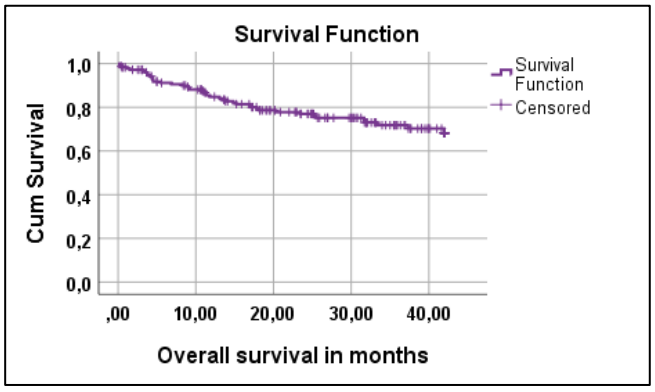


Figure 11a

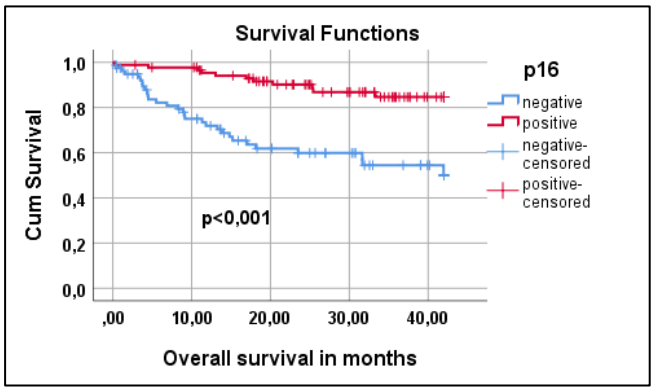


Figure 11b

These Kaplan Meyer survival functions depict the overall survival of the whole study group (Figure 11, purple) and the comparison of overall survival (Figure 11b) between p16-positive (red) and p-16 negative OPSCC (blue).

3.3.2 Progression and Disease-free survival

To describe the outcome of the patient not only the overall survival is of interest but also the duration of survival without a progression of the disease of any kind, in the following described with the term disease-free survival. Several events counted as progress, firstly local recurrence in the oropharynx as well as lymph node recurrence, secondly progression of the primary tumor and occurrence of distant metastasis.

3.3.2.1 Whole study population

In the whole study group (n=178) 117 patients (65,7%) did not have any progression of the disease, 61 patients (34,3%) did have one. The primary manifestation of progress in these 61 patients was local recurrence in 13 patients (21,3%), soft tissue metastasis in 3 patients

(4,9%), distant metastases in 22 patients (36,1%), progression of primary tumor in 10 patients (16,4%) and second malignoma in 13 patients (21,3%).

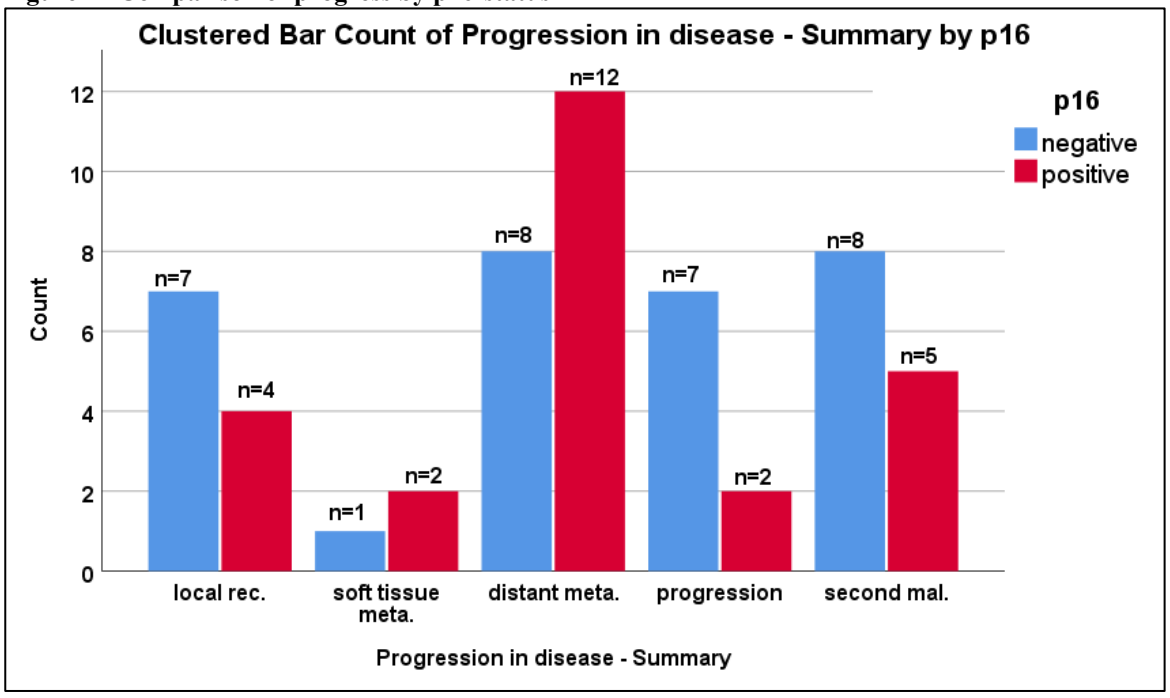
3.3.2.2 p16-negative OPSCC

In the p16-negative group (n= 79) 48 patients (60,8%) did not have any progression of the disease, 31 patients (39,2%) did have one. The primary manifestation of progress in these 31 patients was local recurrence in 7 patients (22,6%), soft tissue metastasis in 1 patient (3,2%), distant metastases in 8 patients (25,8%), progression of primary tumor in 7 patients (22,6%) and second malignoma in 8 patients (25,8%), as outlined in Figure 12.

3.3.2.3 p16-positive OPSCC

In the p16-positive OPSCC group (n=88) 63 patients (71,6%) did not have any progression of the disease, 25 patients (28,4%) did have one. The primary manifestation of progress in these 25 patients was local recurrence in 4 patients (16,0%), soft tissue metastasis in 2 patients (8,0%), distant metastases in 12 patients (48,0%), progression of primary tumor in 2 patients (8,0%) and second malignoma in 5 patients (20,0%), as outlined in Figure 12.

Figure 12 Comparison of progress by p16-status



This clustered bar chart depicts the distribution of kinds of progress for p16-negative (blue) and p16-positive (red) OPSCC. Abbreviations: rec: recurrence, meta: metastasis, mal. malignoma

3.3.2.4 Disease free survival function

To assess the difference in the occurrence of disease progression between the two subgroups of OPSCC, we analyzed the time until a patient showed symptoms of progression.

Analyzing all OPSCC of our study group together, the 1-year DFS rate was 75,4% and the 3-year DFS-rate was 51,3%, the mean DFS was 28,1 months (95% CI: 25,7-30,6).

In p16-positive OPSCC the 1-year DFS-rate was 88,5% and the 3-year DFS-rate was 68,1%, the mean DFS was 33,6 months (95% CI: 30,8-36,4).

In p16-negative OPSCC the 1-year DFS-rate was 62,5% and the 3-year DFS-rate was 33,5%, the mean DFS was 22,1 months (95% CI: 18,2-25,9).

Patients with a p16-negative OPSCC had a worse disease-free survival compared to p16-positive OPSCC and this difference was statistically significant (p<0,001).

Similar to the overall survival, Figure 13a and b show the necessity of treating the two different etiologies of OPSCC as two separate entities, because the distinction between the two groups is beneficial for venturing a prognosis.

Figure 13 DFS a) whole study group b) comparing p16-positive to p16-negative OPSCC

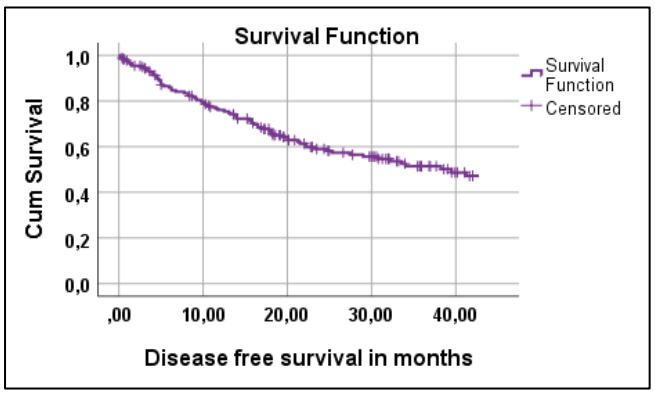


Figure 13a

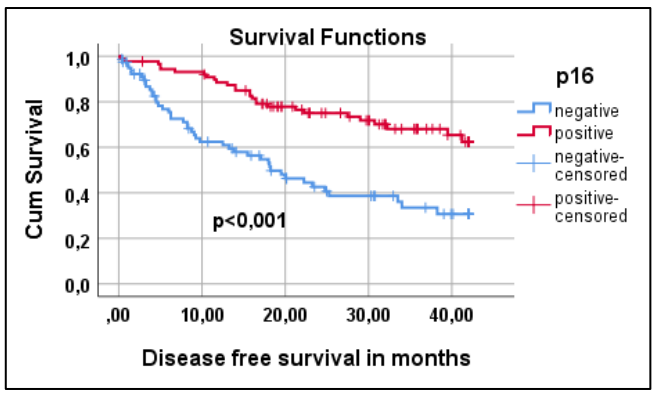


Figure 13b

These Kaplan Meyer survival functions depict the disease-free survival of the whole study group (Figure 13a, purple) and the comparison of disease-free survival (Figure 13b) between p16-positive (red) and p-16 negative OPSCC (blue).

3.4 TNM – Comparison by UICC stages

One important aspect of a staging system is to give a reliable prognosis of the illness. Therefore, for this thesis we examined the data of our patient population and tried to evaluate benefits and inexactness's of the new 8th UICC TNM (1) classification for p16-positive patients compared to the previous 7th UICC TNM classification (27). Therefore, all patients have been classified with both staging manuals for comparison.

As the staging manual for p16-negative OPSCC remains mostly the same in the 8th Edition (1), there was hardly any shift between the stages between the 7th Edition (TNM7) and the 8th Edition (TNM8). The p16-negative patients (n=77) included in this analysis have been classified as stage I (TNM7: n=6; 7,8% vs. TNM8: n=6; 7,8%), stage II (TNM7: n=7; 9,1% vs. TNM8: n=7; 9,1%), stage III (TNM7: n=16; 20,8% vs. TNM8: n=16; 20,8%), stage IVA (TNM7: n=27; 35,1% vs. TNM8: n=25; 32,5%), stage IVB (TNM7: n=11; 14,3% vs. TNM8: n=13; 16,9%) and stage IVC (TNM7: n=10; 13,0% vs. TNM8: n=10; 13,0%).

In contrast, the 8th Edition of the UICC TNM staging manual (1) did change the classification of p16-positive OPSCC, as intended. As a result, there was a noteworthy shift between the stages of the 7th Edition (27) and the 8th Edition. In the p16-positive group (n=88) patients have been classified as stage I (TNM7: n=4; 4,5% vs. TNM8: n=39; 44,3%), stage II (TNM7: n=7; 8,0% vs TNM8: n=19; 21,6%), stage III (TNM7: n=8; 9,1% vs. TNM8: n=27; 30,7). Using the 7th Edition 59 patients (67%) have been classified with stage IVA disease, 7 patients (8,0%) with stage IVB and 3 patients (3,4%) with stage IVC. After applying the p16-positive group to the 8th Edition only 3 (3,4%) patients were left to be classified with stage IV disease, as outlined in Figure 14.

Figure 14 Frequency of UICC stages a) 7th Edition TNM b) 8th Edition TNM

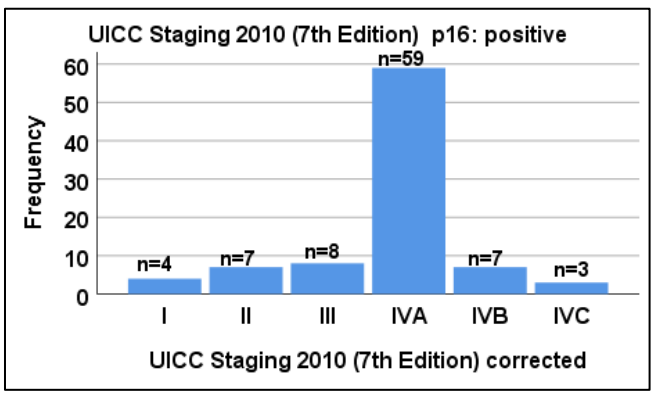


Figure 14a

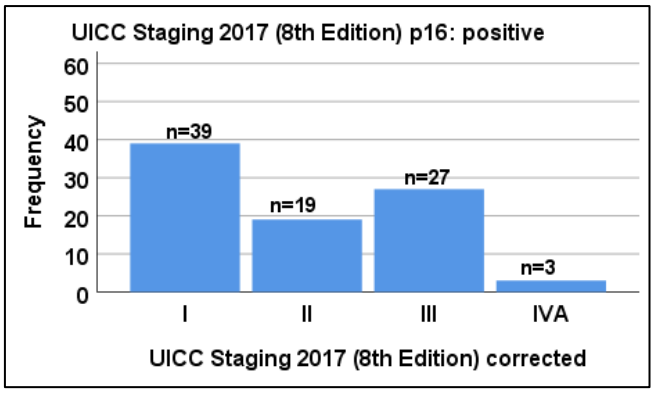


Figure 14b

These bar charts depict the distribution of tumor stages in p16-positive OPSCC according to the 7th Edition (left, Figure 14a) and the 8th Edition (right, Figure 14b) of the TNM classification. Abbreviations: UICC: Union international contre le cancer.

The stage shift of p16-positive OPSCC can be demonstrated more clearly by clustering stage I and II as early-stage disease and stage III and IV as advanced-stage disease. When using the 7th Edition (27) patients were classified with an early-stage disease in 12,5 % vs. 65,9% when applying the 8th Edition (1) and as advanced-stage disease in 87,5% (7th Edition) vs. 34,1 (8th Edition) in the p16-positive group.

3.4.1 Overall survival

3.4.1.1 p16-negative OPSCC

UICC TNM 7th Edition

For the overall survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 7th Edition of TNM, but no significant difference in-between individual stages, in detail. Stage I and stage II disease did not show significant difference (p=0,355), nor did stage II and stage III disease (p=0,776). As shown in Figure 15a OPSCC with stage IVA had a lower overall survival than those with stage III (p=0,081). Stage IVA and stage IVB disease (p= 0,284) did not differ significantly nor did stage IVB and stage IVC disease (p=0,240). The stages were in the expected order as seen in Figure 15a. For patients with stage I carcinoma the 3-year OS-rate was 100%, for stage II it was 86%, for stage III it was 71%, for stage IVA it was 50% and for stage IVC it was 10%. We could not calculate the 3-year OS-rate for IVB disease, but we can report a 1-year OS rate for stage IVB disease of 51%.

UICC TNM 8th Edition

For the overall survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 8th Edition of TNM, but no significant difference in-between individual stages, in detail. Stage I and stage II disease did not show a significant difference (p=0,355), nor did stage II and stage III disease (p=0,776). As shown in Figure 15b OPSCC with stage IVA had a lower overall survival than those with stage III, but without statistical significance (p=0,080). Stage IVA and stage IVB disease (p= 0,567) did not differ significantly nor did stage IVB and stage IVC disease (p=0,139). As the staging manual for p16-negative OPSCC remains mostly the same in the new 8th Edition (1), the graphs, Figure 15a and b, did not differ much. Stages were in the expected order. For patients with a stage I carcinoma the 3-year OS-rate was 100%, for stage II tumors it was 86%, for stage III tumors it was 71%, for stage IVA tumors it was 51%, for IVB tumors it was 41% and for stage IVC tumors it was 10%.

Figure 15 OS of p16-negative OPSCC by UICC stage a) 7th Edition TNM b) 8th Edition TNM

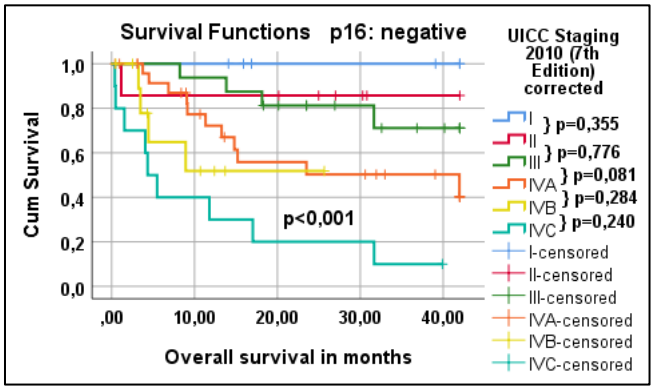


Figure 15a

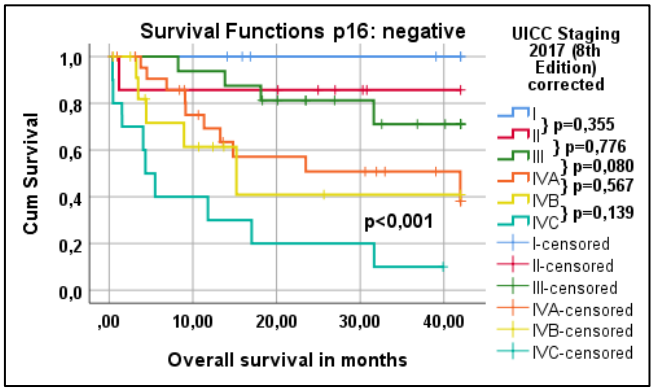


Figure 15b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-negative OPSCC compared by stage according to the 7th Edition (left, Figure 15) and 8th Edition (right, Figure 15b) of TNM classification. Abbreviations: UICC: Union international contre le cancer

3.4.1.2 p16-positive OPSCC

UICC TNM 7th Edition

For the overall survival in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 7th Edition. of TNM. Between individual stages, only stage IVA and IVB were statistically different (p=0,019). Stage I and stage II disease did not have a significant difference (p=0,781), nor did stage II and stage III disease (p=0,285), nor stage III and stage IVA disease (p=0,446) or stage IVB and stage IVC disease (p=0,162).

Unlike the graph above comparing the stages of p16-negative patients (Figure 15) the stages were not in the expected order. According to 7th Edition, Figure 16a showed that patients with advanced disease (stage III or IVA) had a better OS than those with early-stage disease (stage I and II). For patients with stage I carcinoma the 3-year OS-rate was 75%, for stage II it was 86%, for stage III it was 100%, for stage IVA it was 92% and for IVB it is 69%. We could not calculate the 3-year OS-rate for IVC disease, but we can report a 1-year OS rate for stage IVC disease of 33%.

UICC TNM 8th Edition

For the overall survival in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 8th Edition of TNM. Between individual stages, only stage III and IV were statistically different (p<0,001). Stage I and stage II disease did not have a significant difference (p=0,357), nor did stage II and stage III disease (p=0,615). These findings were supported by Figure 16b,

as the curves of stage I, II and III were very similar, only stage IV showing an evident lower overall survival.

This time the stages were in expected order, which means the 8th UICC TNM staging manual (1) gives a more accurate prognosis than its predecessor. The 3-year OS-rate for stage I tumors was 94%, for stage II it was 85% and for stage III it was 81%. We could not calculate the 3-year OS-rate for IV disease, the 1-year OS rate for stage IV disease was 33%. Mean OS for stage I was 40,6 months (95% CI: 38,8-42,5), for stage II it was 38,8 months (95% CI: 34,7-42,9), for stage III 36,97 months (95% CI: 32,1-41,5) and for stage IV 16,3 months (95% CI: 0,0-33,4).

Figure 16 OS of p16-positive OPSCC by UICC stage a) 7th Edition TNM b) 8th Edition TNM

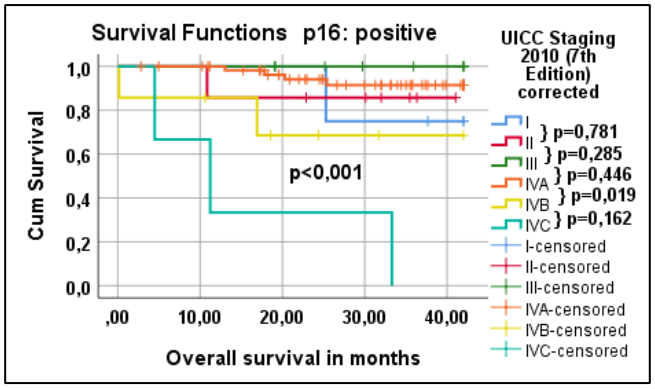


Figure 16a

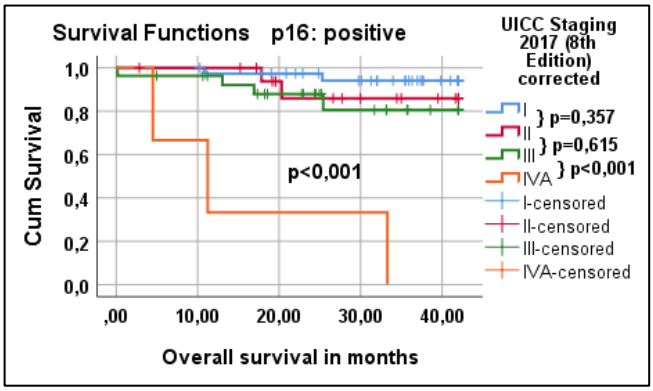


Figure 16b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-positive OPSCC compared by stage according to the 7th Edition (left, Figure 16a) and 8th Edition (right, Figure 16b) of TNM classification. Abbreviations: UICC: Union international contre le cancer

3.4.1.3 Early vs. advanced-stage disease

Due to the small number of patients in stage I and II we decided to group our patients in early-stage (stage I and II) and advanced-stage disease (stage III and IV) and performed another analysis of the overall survival.

p16-negative OPSCC

When using the 7th Edition of UICC TNM classification (27) for p16-negative OPSCC, the 3-year OS-rate for patients with early-stage disease was 92%, for patients with advanced-stage disease it was 47%. Patients with early-stage disease had a mean OS of 38,9 months (95% CI: 32,9-44,8) and patients with advanced-stage disease 26,1 months (95% CI: 21,7-30,5). The difference in survival was statistically significant (Log Rank, p=0,017). When using the 8th Edition of UICC TNM classification (1) the analysis led to the exact same numbers.

p16-positive OPSCC

When using the 7th Edition of UICC TNM classification (27) for p16-positive OPSCC, the 3-year OS-rate for patients with early-stage disease was 81%, whereas for patients with advanced-stage disease it was 85%. Patients with early-stage disease had a mean OS of 37,5 months (95% CI: 31,6-43,4) and patients with advanced-stage disease 38,5 months (95% CI: 36,3-40,7), as outlined in Figure 17a. The difference in survival was not statistically significant (Log Rank, p=0,685)

In contrast, when using the 8th Edition of UICC TNM classification (1) for p16-positive OPSCC, the 3-year OS-rate for patients with early-stage disease was 92%, whereas for patients with an advanced-stage disease it was 67%. Patients with early-stage disease had a mean OS of 40,1 months (95% CI: 38,4-41,9) and patients with advanced-stage disease 34,4 months (95% CI: 29,5-39,4), as outlined in Figure 17b. The difference in survival was statistically significant (Log Rank, p=0,012); this again showed that the 8th Edition of UICC TNM classification (1) led to a more accurate prognosis for patients with p16-positive OPSCC, this time the statement could rely on statistically significant results.

Figure 17 OS of p16-positive OPSCC early vs. advanced-stage disease a) 7th Edition TNM b) 8th Edition TNM

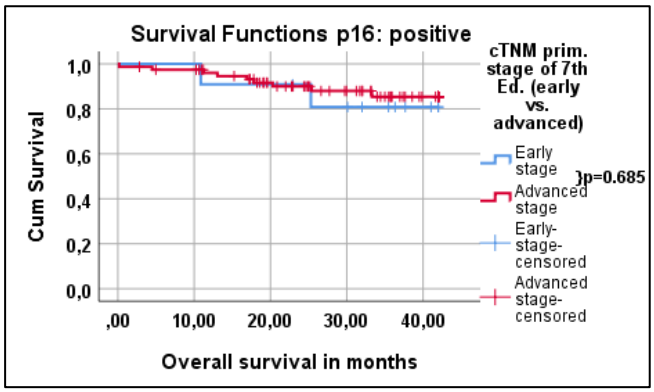


Figure 17a

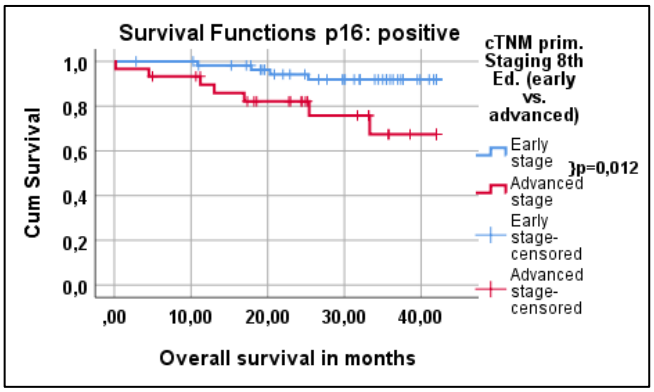


Figure 17b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-positive OPSCC comparing early-stage and advanced-stage disease according to the 7th Edition (left, Figure 17a) and 8th Edition (right, Figure 17b) of TNM classification. (Explanation: early-stage: stage I and II, advanced-stage: stage III and IV)

3.4.2 Disease free survival

For this analysis, an event was defined as local recurrence in the oropharynx as well as lymph node recurrence or progression of the primary tumor or occurrence of distant metastasis or if the patient died of the cancer.

3.4.2.1 p16-negative OPSCC

UICC TNM 7th Edition

For the disease-free survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 7th Edition of TNM. Between individual stages, only stage IVA and IVB were significantly different (p=0,044). Stage I and stage II disease were not significantly different (p=0,958), as well as stage II and stage III disease (p=0,830), and stage III and stage IVA disease (p=0,075) and stage IVB and stage IVC disease (p=0,670). In addition, the stages were not in the expected order, as outlined in Figure 18a. For patients with stage I carcinoma 3-year DFS-rate was 33%, for stage III tumors it was 51%, for stage IVA tumors it was 34% and for stage IVC tumors it was 10%. We could not calculate the 3-year DFS-rate for stage II and IVB disease, but we can report a 1-year DFS-rate of 86% for stage II carcinomas and 13% for stage IVB carcinomas. According to the 7th Edition, p16-negative patients with stage III disease had the longest mean disease-free survival, 31,4 months (95% CI: 25,3-37,6), followed by patients with stage I disease (29,8 months (95% CI: 20,0-39,7)), stage II disease (23,4 months (95% CI: 15,8-31,0)), stage IVA disease (21,6 months (95% CI: 14,8-28,4)), stage IVB disease (6,8 months (95% CI: 5,1-8,6)) and stage IVC disease (8,6 months (95% CI: 18,4-26.2)).

UICC TNM 8th Edition

For the disease-free survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 8th Edition of TNM, but no significant difference in-between individual stages, in detail. Stage I and stage II disease did not show a significant difference (p=0,958), nor did stage II and stage III disease (p=0,830), nor stage III and stage IVA disease (p=0,060), nor stage IVA and stage IVB disease (p= 0,137) or stage IVB and stage IVC disease (p=0,456).

As the staging manual for p16-negative OPSCC remained mostly the same in the new 8th Edition, the graphs did not differ much (Figure 18a and b). However, the stages were not in the expected order. For patients with stage I carcinoma 3-year DFS-rate was 33%, for stage III tumors it was 51%, for stage IVA tumors it was 32%, for stage IVC tumors it was 10%. We could not calculate the 3-year DFS-rate for stage II and IVB disease, but we can report

a 1-year DFS-rate for stage II carcinomas of 86% and for stage IV disease of 13%. Patients with stage III disease had the longest mean DFS, 31,4 months (95% CI: 25,3-37,6), followed by patients with stage I disease (29,8 months (95% CI: 20,0-39,7)), stage II disease (23,4 months (95% CI: 15,8-31,0)), stage IVA disease (21,4 months (95% CI: 14,5-28,3)), stage IVB disease (13,3 months (95% CI: 3,9-22,7)) and stage IVC disease (8,6 months (95% CI: 18,4-26.2)).

Figure 18 DFS of p16-negative OPSCC by UICC stage a) 7th Edition TNM b) 8th Edition TNM

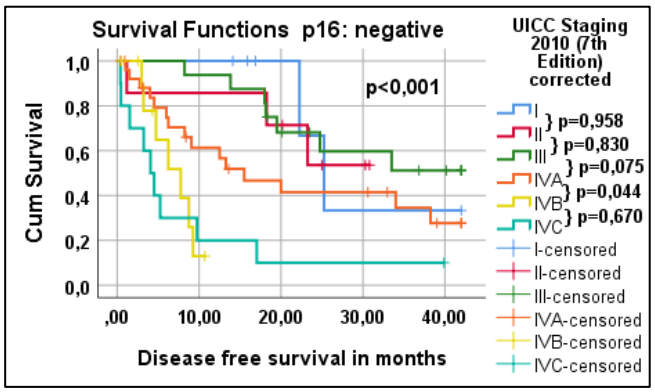


Figure 18a

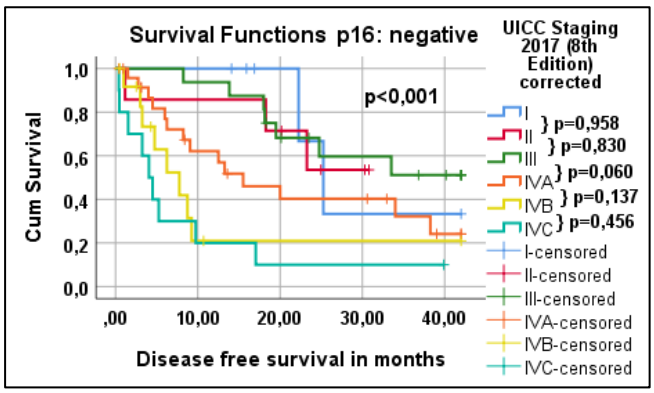


Figure 18b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-negative OPSCC compared by stage according to the 7th Edition (left, Figure 18a) and 8th Edition (right, Figure 18b) of TNM classification. Abbreviations: UICC: Union international contre le cancer

3.4.2.2 p16-positive OPSCC

UICC TNM 7th Edition

For the DFS in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 7th Edition of TNM. In-between individual stages, stage IVA and IVB were statistically different (p=0,043) same as stage IVB and stage IVC (p=0,022). Stage I and stage II disease did not show a significant difference (p=0,494), nor did stage II and stage III disease (p=0,881) or stage III and stage IVA disease (p=0,361). The curves for stage I, II, III and IVA did not differ much, as outlined in Figure 19. In addition, the stages were not in the expected order for sufficient prognosis. According to the 7th Edition p16-positive patients with stage I carcinoma had a 3-year DFS-rate of 75%, for stage II 86%, for stage III tumors 86%, for stage IVA 68% and for stage IVB 57%. We could neither calculate the 1-year DFS-rate nor the 3-year DFS-rate for stage IVC tumors. Patients with stage III disease showed the longest mean DFS, 38,8 months (95% CI: 33,0-44,7), followed by patients with stage II disease (36,7 months (95% CI: 27,1-46,3)), stage I disease (36,5 months (95% CI: 27,9-45,2)), stage IVA disease (34,7 months (95% CI: 31,5-37,8)), stage IVB disease (26,1 months (95% CI: 12,5-39,7)) and stage IVC disease (5,3 months (95% CI: 0,0-10,6)).

UICC TNM 8th Edition

For the DFS in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all stages for classification by 8th Edition of TNM. In-between individual stages, only stage III and IV were statistically different (p<0,001)). Stage I and stage II disease did not show significant difference (p=0,472), nor did stage II and stage III disease (p=0,471). Compared to UICC TNM 7th Edition (27) the stages in UICC TNM 8th Edition (1) were in expected order, meaning that the 8th Edition (Figure 19b) allowed to give a more accurate prognosis than its predecessor. For patients with stage I carcinoma the 3-year DFS-rate was 80%, for stage II it was 59% and for stage III it was 63%. We could neither calculate the 1-year DFS-rate nor the 3-year DFS-rate for stage IV tumors. The mean DFS for stage I was 37,3 months (95% CI: 34,2-40,4), for stage II it was 34,5 months (95% CI: 29.0-40,0), for stage III 30,8 months (95% CI: 25,1-36,4) and for stage IV 5,3 months (95% CI: 0,0-10,6).

Figure 19 DFS of p16-positive OPSCC by UICC stage a) 7th Edition TNM b) 8th Edition TNM

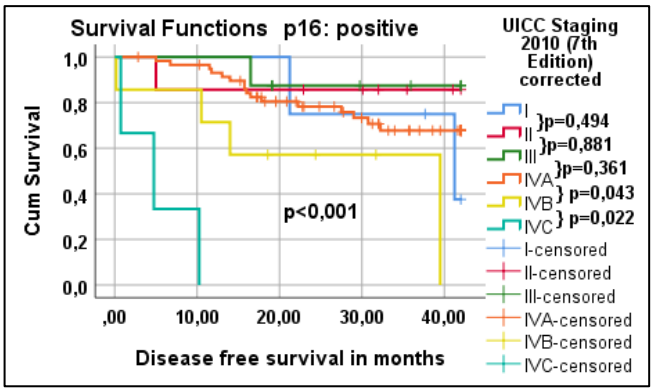


Figure 19a

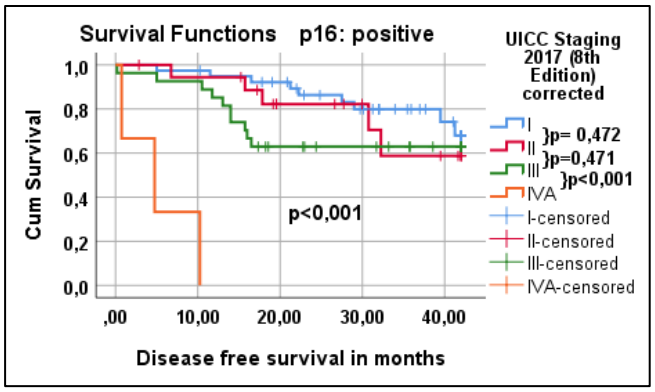


Figure 19b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-positive OPSCC compared by stage according to the 7th Edition (left, Figure 19a) and 8th Edition (right, Figure 19b) of TNM classification. Abbreviations: UICC: Union international contre le cancer

3.4.2.1 Early vs. advanced-stage disease

Due to the small number of patients in stage I and II we decided to group our patients in early-stage (stage I and II) and advanced-stage disease (stage III and IV) and performed further analysis.

p16-negative OPSCC

When using the 7th Edition of UICC TNM classification (27) for p16-negative OPSCC, the 3-year DFS-rate for patients with early-stage disease was 44%, whereas for patients with advanced-stage disease it was 31%. Patients with early-stage disease had a mean DFS of 29,5 months (95% CI: 21,5-37,4) and patients with advanced-stage disease 20,5 months

(95% CI: 16,2-24,8). The difference in survival was not statistically significant (Log Rank, p=0,093). When using the 8th Edition of UICC TNM classification (1) the analysis led to the exact same numbers.

p16-positive OPSCC

When using the 7th Edition of UICC TNM classification (27) for p16-positive OPSCC, the 3-year DFS-rate for patients with early-stage disease was 82%, whereas for patients with advanced-stage disease it was 66%. Patients with early-stage disease had a mean DFS of 36,5 months (95% CI: 29,7-43,4) and patients with advanced-stage disease 33,1 months (95% CI: 30,1-36,3), as outlined in Figure 20a. The difference in survival was not statistically significant (Log Rank, p=0,632).

In contrast, when using the 8th Edition of UICC TNM classification (1) for p16-positive OPSCC, the 3-year DFS-rate for patients with early-stage disease was 74%, whereas for patients with an advanced-stage disease it was 57%. Patients with early-stage disease had a mean DFS of 36,5 months (95% CI: 33,7-39,3) and patients with advanced-stage disease 28,2 months (95% CI: 22,4-34,0), as outlined in Figure 20b. The difference in survival was statistically significant (Log Rank, p=0,025).

This again showed that the 8th Edition of UICC TNM classification (1) led to a more accurate prognosis for patients with p16-positive OPSCC, this time the statement could rely on statistically significant results.

Figure 20 DFS of p16-positive OPSCC early vs. advanced-stage disease a) 7th Edition TNM b) 8th Edition

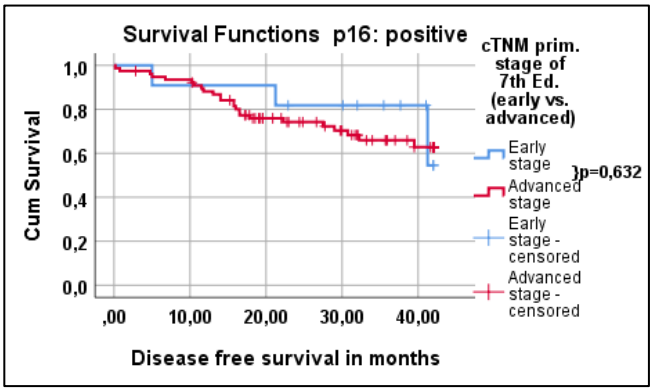


Figure 20a

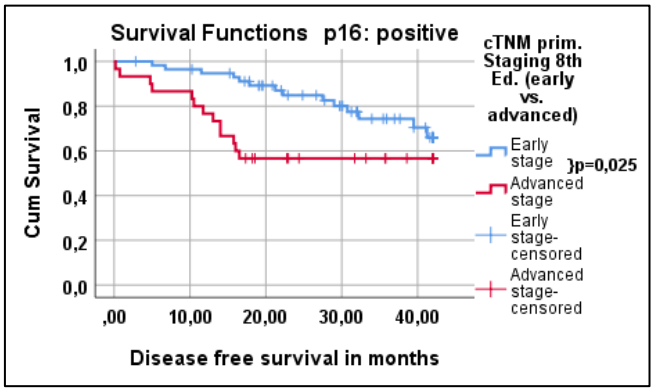


Figure 20b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-positive OPSCC comparing early-stage and advanced-stage disease according to the 7th Edition (left, Figure 20a) and 8th Edition (right, Figure 20b) of UICC TNM classification. (Explanation: early-stage: stage I and II, advanced-stage: stage III and IV)

3.5 TNM- Comparison by T and N categories

As described in the chapters before, our analyses did not lead to many significant differences between the individual stages of the 7th Edition (27) and 8th Edition of UICC TNM classification (1), significant differences were found when clustering patients in early-stage and advanced-stage diseases only. Therefore, we reevaluated OS and DFS, comparing the survival by the different T and N categories separately. This could indicate which TNM staging element should be evaluated further to improve its predictive value.

3.5.1 T categories-Overall survival

3.5.1.1 p16-negative OPSCC

For the overall survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference among all T categories of both the 7th Edition of TNM (Log Rank, p<0,001) and the 8th Edition of TNM (Log Rank, p<0,001). However, between individual stages, the only statistically significant difference could be found between T3 and T4 using the 7th Edition of UICC TNM classification (27) (p=0,039). Applying the 8th Edition of UICC TNM classification (1) T4 also had a lower overall survival than T3 as shown in Figure 21b, but without statistical significance (p=0,056). Thus, it appeared as if patients with T2 OPSCC had the longest overall survival, as seen in Figure 21a and b.

Figure 21 OS of p16-negative OPSCC by T category a) 7th Edition TNM b) 8th Edition TNM

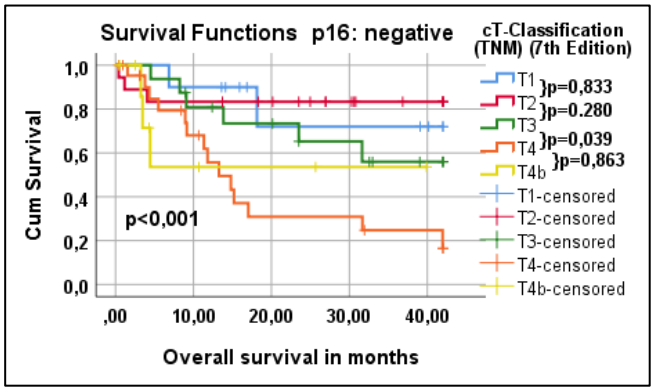


Figure 21a

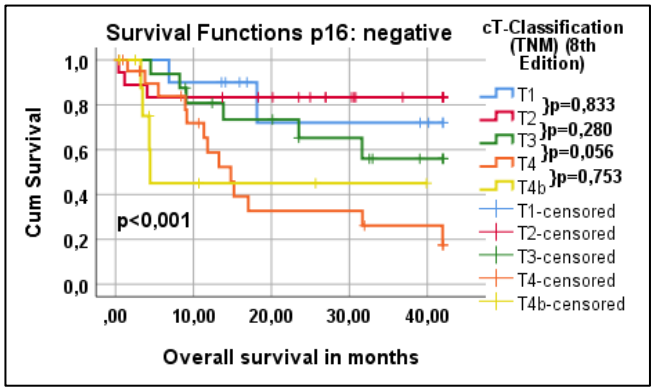


Figure 21b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-negative OPSCC compared by T categories according to the 7th Edition (left, Figure 21a) and 8th Edition (right, Figure 21b) of UICC TNM classification.

3.5.1.2 p16-positive OPSCC

For the overall survival in p16-positive OPSCC, Kaplan Meyer did not show a statistically significant difference among all T categories of the 7th Edition of TNM (Log Rank, p=0,156) and the 8th Edition of TNM (Log Rank, p=0,446). Furthermore, differences between adjacent T categories were not statistically significant. The Kaplan Meyer survival functions (Figure 22a and b) showed that the curves of T1 and T2 were almost identical in p16-positive patients. The same applied to T3 and T4, this was true for both 7th (27) and 8th Edition of UICC TNM classification (1). The only difference was suspected between T4a and T4b as outlined in Figure 22a, this difference was statistically not significant (p=0,169).

Figure 22 OS of p16-positive OPSCC by T category a) 7th Edition TNM b) 8th Edition TNM

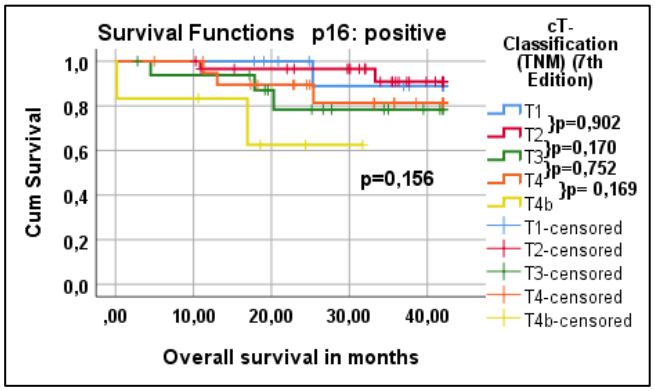


Figure 22a

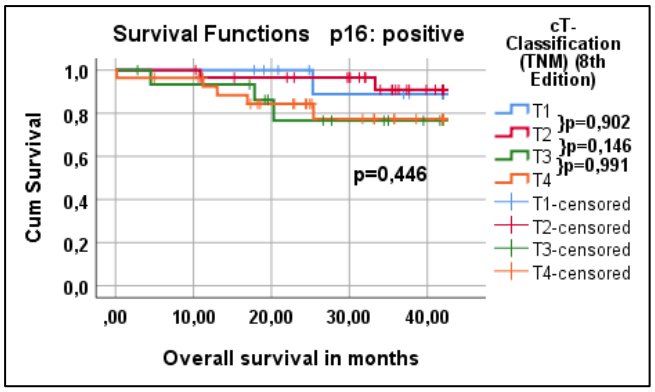


Figure 22b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-positive OPSCC compared by T categories according to the 7th Edition (left, Figure 22a) and 8th Edition (right, Figure 22b) of UICC TNM classification.

3.5.2 T categories- Disease-free survival

3.5.2.1 p16-negative OPSCC

For the disease-free survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference among all T categories of both the 7th Edition of TNM (Log Rank, p<0,001) and the 8th Edition of TNM (Log Rank, p<0,001). The only statistically significant difference could be found between T3 and T4 using the 7th Edition of UICC TNM classification (27) (p=0,36). Applying the 8th Edition of UICC TNM classification (1) T4 also had a lower disease-free survival than T3 as shown in Figure 23b, but without statistical significance (p=0,052).

As outlined in Figure 23a and b, patients with a T2 OPSCC staging showed the longest disease-free survival.

Figure 23 DFS of p16-negative OPSCC by T category a) 7th Edition TNM b) 8th Edition TNM

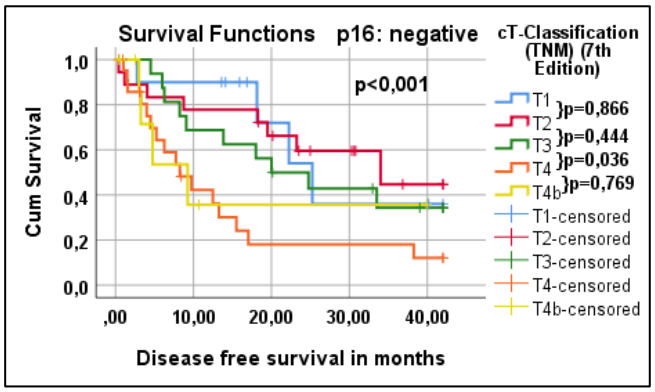


Figure 23a

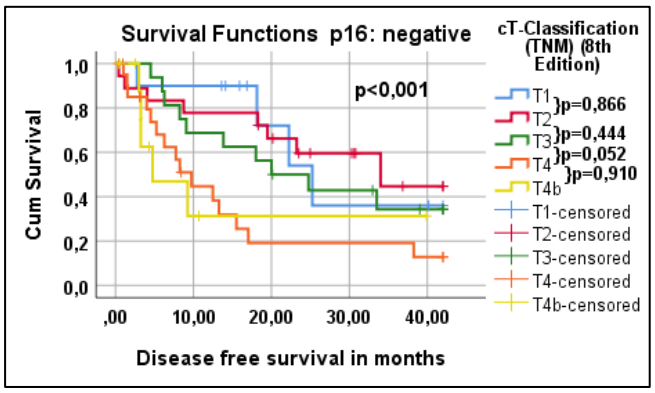


Figure 23b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-negative OPSCC compared by T categories according to the 7th Edition (left, Figure 23a) and 8th Edition (right, Figure 23b) of UICC TNM classification.

3.5.2.2 p16-positive OPSCC

For the disease-free survival in p16-positive OPSCC, Kaplan Meyer did not show a statistically significant difference among all T categories of both the 7th Edition of TNM (Log Rank, p=0,238) and the 8th Edition of TNM (Log Rank, p=0,316). Furthermore, differences between adjacent T categories were not statistically significant.

T1-OPSCC had the longest disease-free survival followed by those with T2-OPSCC as shown in Figure 24a; further when applying the 7th Edition of UICC TNM classification (27) patients with T4-OPSCC had a longer disease-free survival compared to patients with T3-OPSCC. This changed when using the 8th Edition (1), showing almost identical disease-free survival functions for both T3- and T4-OPSCC, as shown in Figure 24b.

Figure 24 DFS of p16-positive OPSCC by T category a) 7th Edition TNM b) 8th Edition TNM

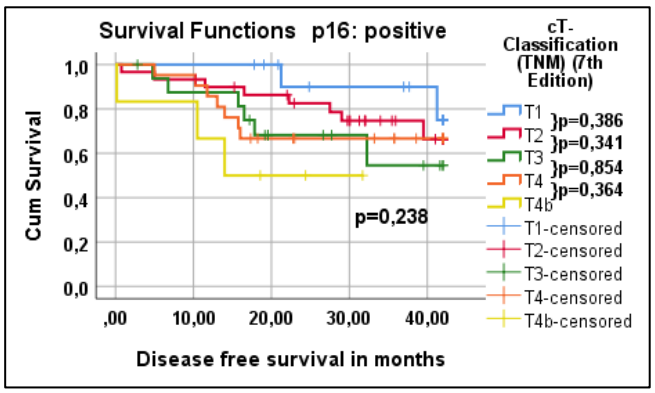


Figure 24a

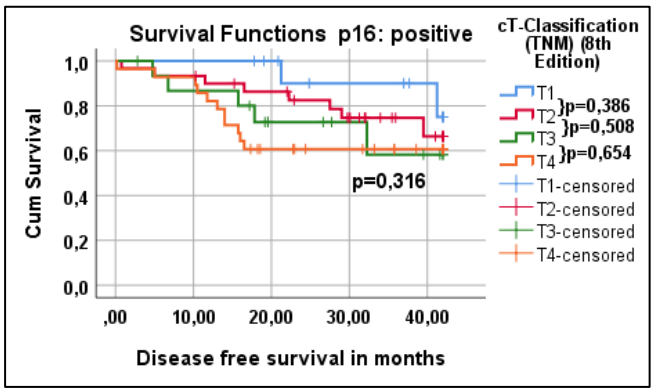


Figure 24b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-positive OPSCC compared by T categories according to the 7th Edition (left, Figure 24a) and 8th Edition (right, Figure 24b) of UICC TNM classification.

3.5.3 N categories-Overall survival

3.5.3.1 p16-negative OPSCC

For the OS in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference among all N categories of the 7th Edition of TNM (Log Rank, p=0,009) and the 8th Edition of TNM (Log Rank, p=0,009). Differences between adjacent N categories were not statistically significant neither with the 7th Edition nor with the 8th Edition. Applying the 7th Edition of UICC TNM classification (27) the Kaplan Meyer survival functions (Figure 25a) showed that the curves of N0 and N1 proceed similarly, the same applied to N2b, N2c and N3a.

The 8th Edition (1) seemed to be better discriminating between N categories compared to its predecessor, as there were more difference shown graphically between N2b, N2c, N3a and N3b (Figure 25b), however these impressions were not supported by significant results in-between each category.

Figure 25 OS of p16-negative OPSCC by N category a) 7th Edition TNM b) 8th Edition TNM

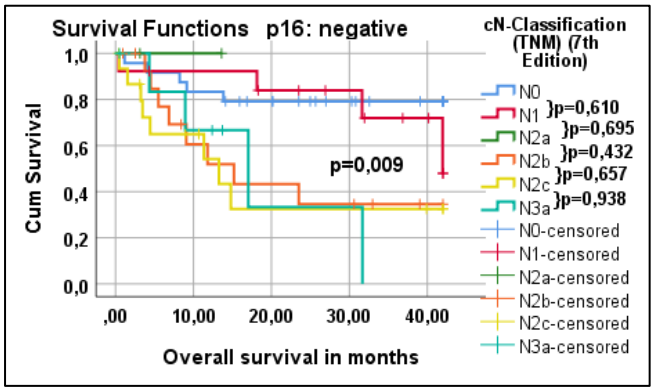


Figure 25a

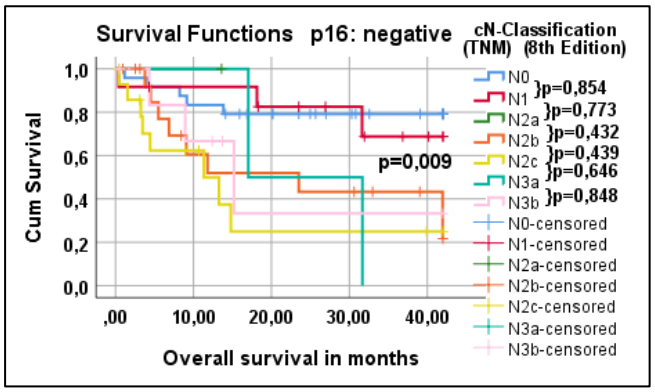


Figure 25b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-negative OPSCC compared by N categories according to the 7th Edition (left, Figure 25a) and 8th Edition (right, Figure 25b) of UICC TNM classification.

3.5.3.2 p16-positive OPSCC

For the overall survival in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference among all N categories of both the 7th Edition of TNM (Log Rank, p=0,005) and the 8th Edition of TNM (Log Rank, p<0,001). However, when applying the 7th Edition of UICC TNM classification (27) there were no significant differences between adjacent stages; patients with N3 tumors had no significant inferior survival as shown in Figure 26a. The only significant difference could be found between N2 and N3 using the 8th Edition (1) (p=0,002). Figure 26b also showed improvement of prognosis for overall survival in N categories.

Figure 26 OS of p16-positive OPSCC by N category a) 7th Edition TNM b) 8th Edition TNM

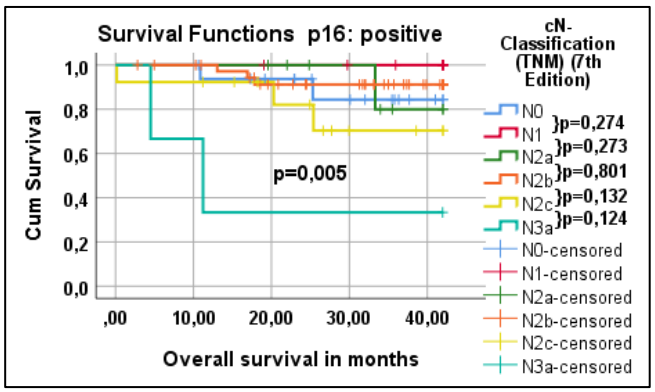


Figure 26a

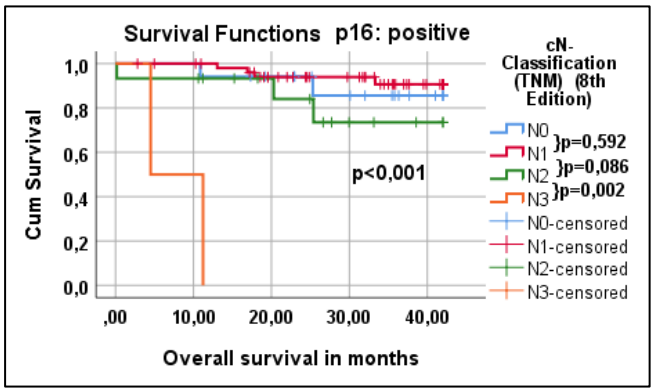


Figure 26b

These Kaplan Meyer survival functions depict the overall survival of patients with p16-positive OPSCC compared by N categories according to the 7th Edition (left, Figure 26a) and 8th Edition (right, Figure 26b) of UICC TNM classification.

3.5.4 N categories- Disease-free survival

3.5.4.1 p16-negative OPSCC

The disease-free survival in p16-negative OPSCC showed statistically significant difference among all N categories for both the 7th Edition of TNM (Log Rank, p=0,004) and the 8th Edition of TNM (Log Rank, p=0,019). Differences between adjacent N categories were not statistically significant neither with the 7th Edition (27) nor with the 8th Edition (1). An apparent inferior disease-free survival could not be observed when comparing adjacent N categories, as outlined in Figure 27a and Figure 27b. Patients with N1 disease seemed to have the longest disease-free survival, this was true for both the7th and 8th Edition of UICC TNM classification.

Figure 27 DFS of p16-negative OPSCC by N category a) 7th Edition TNM b) 8th Edition TNM

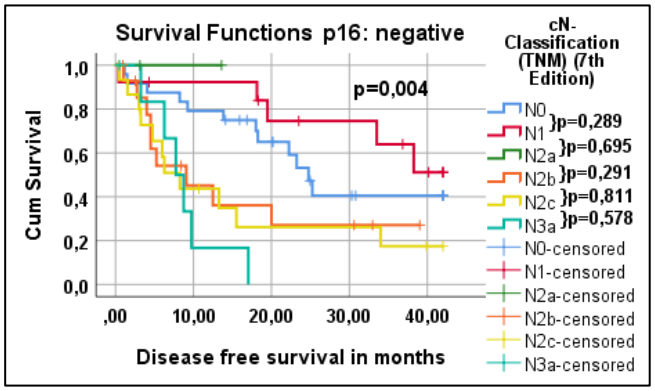


Figure 27a

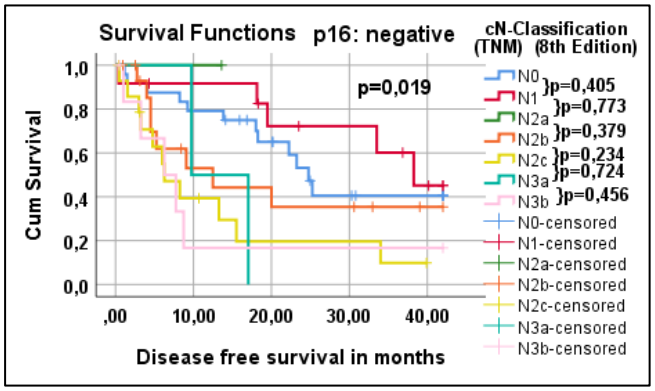


Figure 27b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-negative OPSCC compared by N categories according to the 7th Edition (left, Figure 27a) and 8th Edition (right, Figure 27b) of UICC TNM classification.

3.5.4.2 p16-positive OPSCC

The disease-free survival in p16-positive OPSCC showed statistically significant difference among all N categories of both the 7th Edition of TNM (Log Rank, p=0,008) and the 8th Edition of TNM (Log Rank, p<0,001).When applying the 7th Edition of UICC TNM classification (27) there were no significant differences between adjacent stages, graphically patients with N3 tumors had an inferior survival as shown in Figure 28a. When applying the 8th Edition of UICC TNM (1) classification there could be found statistically significant differences between N1 and N2 (p=0,047) and between N2 and N3 (p=0,006).

Figure 28 DFS of p16-positive OPSCC by N category a) 7th Edition TNM b) 8th Edition TNM

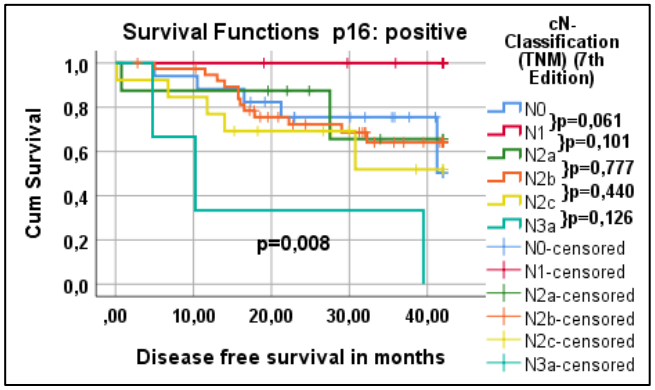


Figure 28a

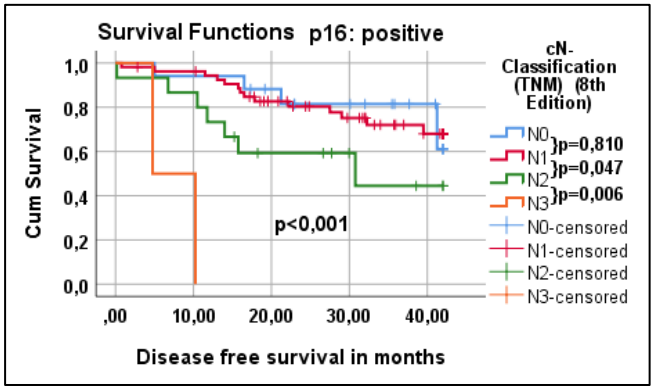


Figure 28b

These Kaplan Meyer survival functions depict the disease-free survival of patients with p16-positive OPSCC compared by N categories according to the 7th Edition (left, Figure 28a) and 8th Edition (right, Figure 28b) of UICC TNM classification

3.5.5 Influence of T respectively N categories on the result of the first restaging

Overall survival and disease-free survival depict the outcome of a patient in a long-term way; however, the results of the first restaging are of great interest as they can indicate the initial success of therapy. Therefore, for the first restaging patients are evaluated three months after the end of their initial treatment, both clinical and imaging aspects are considered. Patients can be categorized as CR (complete remission), PR (part remission), SD (steady disease) or PD (progressive disease).

To further assess the influence of tumor size (T category) or lymph node metastasis (N category) on the outcome of p16-positive patients, the first restaging results were analyzed. Patients were pooled as early-stage disease (stage I and II) respectively advanced-stage disease (stage III and IV).

3.5.5.1 T categories

The analysis of T categories of p16-positive OPSCC showed that patients with T1, T2 and T3 tumors presented with CR in more than 80% at the first restaging. Also, the rates for PR were similar for each subgroup, approximately 10 percent, respectively. Patients with T4 tumors were in complete remission in about two thirds of the cases.

3.5.5.2 N categories

The analysis of patients with p16-positive early-stage disease showed that patients with N0 and N1 were in complete remission in over 80%. Patients with N2 disease presented with CR in 50% and PR in 50% of the cases, as outlined in Figure 29a.

The analysis of patients with p16-positive Advanced-stage disease showed that patients with N0 were in CR in around 60%, patients with N1 in about 70%, and with N2 in 100%. Patients with N3 disease presented PR in 100%, as outlined in Figure 29b.

Figure 29 Comparison of result of 1.st restaging by N category

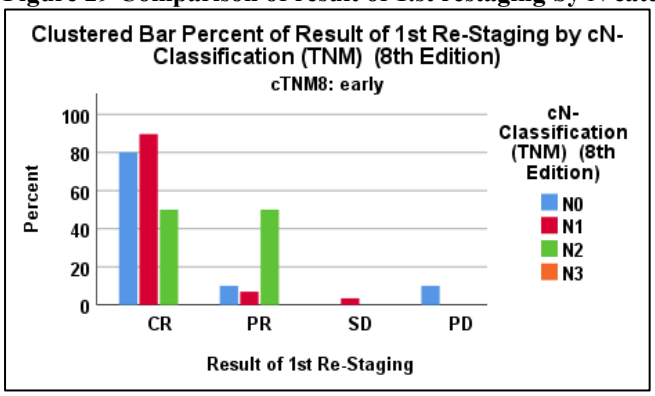


Figure 29a

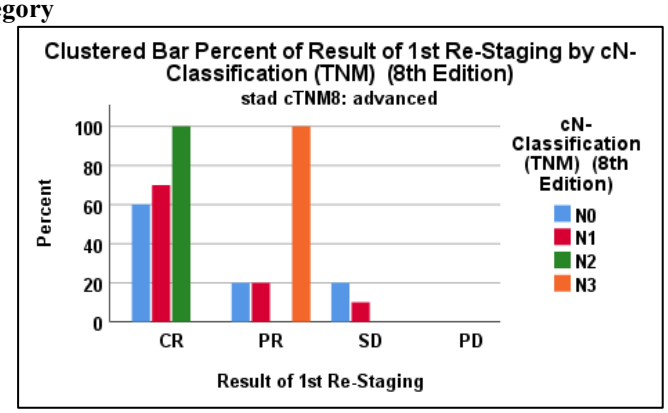


Figure 29b

Left Figure 29a: This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive early-stage disease (stage I and II) with N0 disease (blue), N1 disease (red), N2 disease (green) and N3 disease (orange).

Right Figure 29b: This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive advanced-stage disease (stage III and IV) with N0 disease (blue), N1 disease (red), N2 disease (green) and N3 disease (orange).

Abbreviations: CR: complete remission, PR: part remission, SD: steady disease, PD: progressive disease

3.6 Implementation of the 8th Edition of UICC TNM classification

Even though the 8th Version of UICC TNM classification (1) was released in 2017, it should not be implemented until the January 1st 2018 (20). Therefore, we evaluated the transition time, assessing if implementing the new staging manual in the Head and Neck Tumor Board could lead to adjustment difficulties. At first, we thought of only evaluating the patients classified in the period of 01.01.2018-28.02.2018. Due to the low number of patients and the resulting expected lack of significant results we then evaluated the staging of all patients.

As briefly described in chapter 2.3.2., every patient in our study cohort was staged three times. Patients before 2018 were staged twice according to the 7th Edition of UICC TNM classification (27), one original staging done by the Head and Neck Tumor Board members and one retrospectively by the study team. In addition we classified them also the way they would have been when applying the 8th Edition (1).

The same applies to patients diagnosed after 2018, receiving two versions of staging (official vs. retrospective) according the 8th Edition of UICC TNM (1) and one classification according the 7th Edition UICC TNM (27).

For the evaluation patients were clustered in two groups, those who were classified before 2018 and those after 2018.

3.6.1 Group before 2018

This group includes all patients who were classified before 2018 according to the 7th Edition of UICC TNM classification (27).

3.6.1.1 p16-negative

As seen in Table 13, there were no deviations in stage I and IVC. Of the four patients who were initially been staged with stage II disease, one was upstaged to stage III. Of the eleven patients who were initially been staged with stage III disease, two were down-staged patients to stage II and two patients were upstaged to stage IVA. Of the 29 patients originally staged as IVA two patients were downstaged to stage III and six patients upstaged to stage IVB and one to stage IVC. However, the differences between the two staging-modalities were not statistically significant (Sig.2-tailed, p=0,090).

Table 13 Comparison of staging: original vs. corrected <2018 p16-negative OPSCC

Staging in original TB * Staging corrected according to period Crosstabulation [a]

Count

		Staging corrected according to period						
		I	II	III	IVA	IVB	IVC	Total
Staging in original TB	I	4	0	0	0	0	0	4
	II	0	3	**1**	0	0	0	4
	III	0	**2**	7	**2**	0	0	11
	IVA	0	0	**2**	20	**6**	**1**	29
	IVC	0	0	0	0	0	8	8
Total		4	5	10	22	6	9	56

a. Group = <2018, p16 = negative

This crosstabulation shows the deviations between the original staging from the Head and Neck Tumor Board and the retrospective staging for patients with p16-negative OPSCC staged before 2018. Divergences are written in bold. Abbreviations: TB standing for Head and Neck Tumor Board.

3.6.1.2 p16-positive

As seen in Table 14, there were no deviations in stage I, II, IVB and IVC. Of the twelve patients who were originally staged with stage III disease, one patient was downstaged to stage II and seven patients were upstaged to stage IVA. Of the 45 patients originally staged as IVA two patients were downstaged to stage III and four patients were upstaged to stage IVB. However, the differences between the two staging-modalities were not statistically significant (Sig.2-tailed, p=0,031).

Table 14 Comparison of staging: original vs. corrected <2018 p16-positive OPSCC

Staging in original TB * Staging corrected according to period Crosstabulation [a]

Count

		Staging corrected according to period						
		I	II	III	IVA	IVB	IVC	Total
Staging in original TB	I	4	0	0	0	0	0	4
	II	0	5	0	0	0	0	5
	III	0	**1**	4	**7**	0	0	12
	IVA	0	0	**2**	39	**4**	0	45
	IVB	0	0	0	0	1	0	1
	IVC	0	0	0	0	0	2	2
Total		4	6	6	46	5	2	69

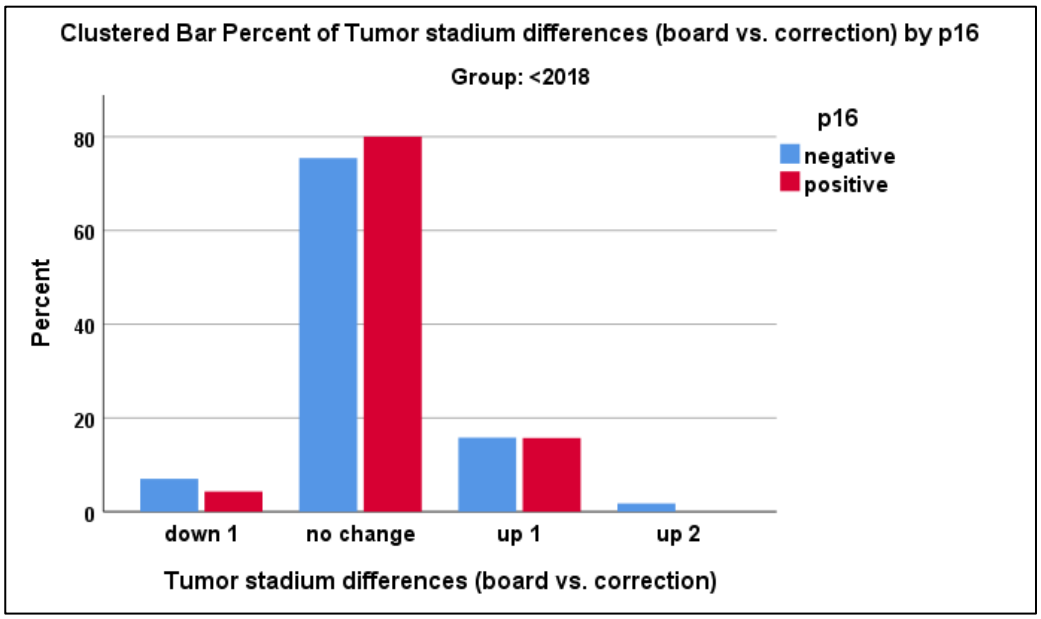
a. Group = <2018, p16 = positive

This crosstabulation shows the deviations between the original staging from the Head and Neck Tumor Board and the retrospective staging for patients with p16-positive OPSCC staged before 2018. Divergences are written in bold. Abbreviations: TB standing for Head and Neck Tumor Board.

3.6.1.3 Comparison p16-negative/ p16-positive before 2018

In order to compare the tumor stadium differences between p16-negative and p16-positive OPSCC for the group "<2018" the bar chart below (Figure 30) was created. It depicted that in both groups in most cases there was no difference in the staging from the Head and Neck Tumor Board compared to the retrospective staging. It seemed like the deviation from the original staging represents only a more minor part of the cases and concerned both p16-negative and positive OPSCC similarly.

Figure 30 Comparison of tumor stadium differences between p16-positive/p16-negative OPSCC, <2018



This bar chart depicts the percentage of tumor stadium differences (original staging from the Head and Neck Tumor Board vs. retrospective staging) in the group < 2018, comparing these percentages between the p16-negative (blue) and p16-positive (red) subgroups. No change means that there was no difference between the original staging and the retrospective one, down 1 means the patients have been down staged one stage, likewise up 1 means the patients have been upstaged one stage.

3.6.2 Group after 2018

This group includes all patients classified after 2018 according to the 8th Edition of UICC TNM classification (1).

3.6.2.1 p16-negative

As seen below (Table 15), there were no deviations in stage I and stage III. Of the five patients who were originally staged with stage II disease, one patient was downstaged to stage I and two patients were upstaged to stage III. Of the ten patients originally staged as IVA two patients were downstaged to stage III and four patients were upstaged to stage IVB. However, the differences between the two staging-modalities were not statistically significant (Sig.2-tailed, p=0,331).

Table 15 Comparison of staging: original vs. corrected >= 2018 p16-negative OPSCC

Staging in original TB * Staging corrected according to period Crosstabulation [a]

Count

		Staging corrected according to period					
		I	II	III	IVA	IVB	Total
Staging in original TB	I	1	0	0	0	0	1
	II	**1**	2	**2**	0	0	5
	III	0	0	2	0	0	2
	IVA	0	0	**2**	4	**4**	10
Total		2	2	6	4	4	18

a. Group = >=2018, p16 = negative

This crosstabulation shows the deviations between the original staging from the Head and Neck Tumor Board and the retrospective staging for patients with p16-negative OPSCC staged after 2018. Divergences are written in bold. Abbreviations: TB standing for Head and Neck Tumor Board.

3.6.2.2 p16-positive

As seen below (Table 16), there were no deviations in stage III. Of the four patients who were originally staged with stage I disease, one patient was upstaged to stage III. Of the seven patients who were originally staged with stage II disease, three patients were upstaged to stage III. Of the 2 patients originally staged as IVA one patient was downstaged to stage I. However, the differences between the two staging- modalities were not statistically significant (Sig.2-tailed, p=0,631).

Table 16 Comparison of staging: original vs. corrected >= 2018 p16-positive OPSCC

Staging in original TB * Staging corrected according to period Crosstabulation [a]

Count

		Staging corrected according to period				
		I	II	III	IVA	Total
Staging in original TB	I	3	0	**1**	0	4
	II	0	4	**3**	0	7
	III	0	0	3	0	3
	IVA	**1**	0	0	1	2
Total		4	4	7	1	16

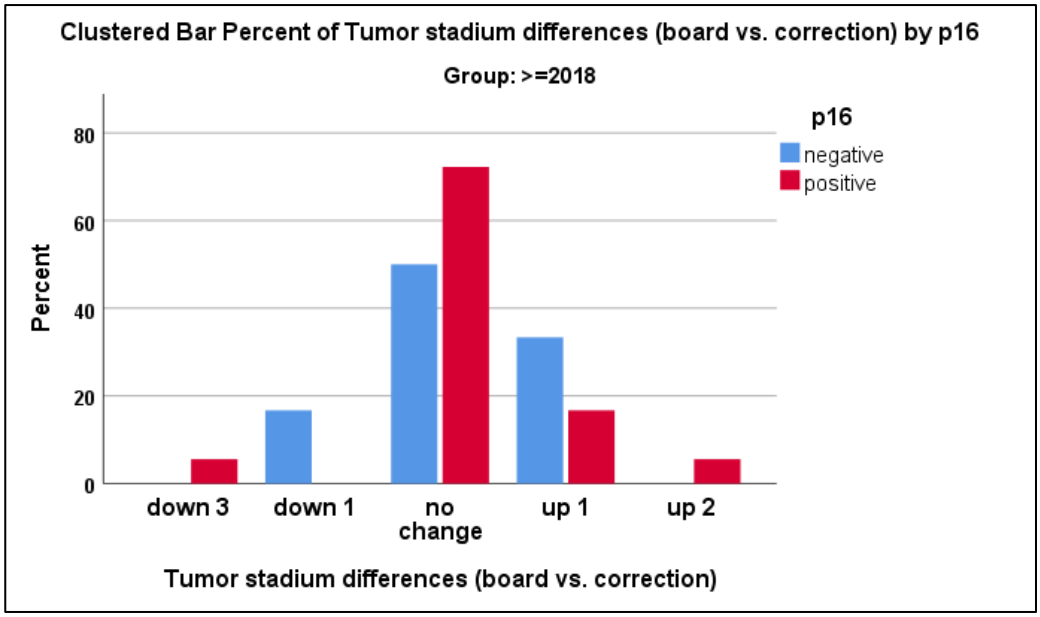
a. Group = >=2018, p16 = positive

This crosstabulation shows the deviations between the original staging from the Head and Neck Tumor Board and the retrospective staging for patients with p16-positive OPSCC staged after 2018. Divergences are written in bold. Abbreviations: TB standing for Head and Neck Tumor Board.

3.6.2.3 Comparison p16-negative/ p16-positive after 2018

In order to compare the tumor stadium differences between p16-negative and p16-positive OPSCC for the group "≥ 2018" the bar chart below (Figure 31) was created. It depicts that in both groups the biggest part was represented by the cases without differences in the staging from the Head and Neck Tumor Board compared to the retrospective one. In the p16-negativ subgroup there were 45% without change and 55% with differences, but these cases alternated just one stage up or down. In the p16-positive subgroup in 72,2% of the cases there was no difference which means in 27,8% there was a change. This portion was much smaller than in the p16-negative subgroup, but in the p16-positive subgroup the cases variated more. This more extensive range of variation in the p16-positive subgroup could be explained by the utilization of the new 8th Edition of UICC TNM classification (1) and possible minor adjustment difficulties. However, it is worth mentioning that these 27,8% who show a difference consisted only of 5 people and as described in chapter 3.6.2.2 above, and the differences between the two staging modalities showed no statistical significance. Even tough, the range of variation in Figure 31 is more extensive compared to the chart of the group before 2018 (Figure 30). It should be noted that the tumor stadium differences in the group staged after 2018 were not statistically significant (Sig.2-tailed p=0,245).

Figure 31 Comparison of tumor stadium differences between p16-positive /p16-negative OPSCC, >=2018



This bar chart depicts the percentage of tumor stadium differences (original staging from the Head and Neck Tumor Board vs. retrospective staging) in the group ≥ 2018, comparing these percentages between the p16-negative (blue) and p16-positive (red) subgroups. No change means that there was no difference between the original staging and the retrospective one, down 1 means the patients have been down staged one stage, likewise up 1 means the patients have been upstaged one stage.

3.7 Therapy

The following chapter describes the treatments that the patients received and how the different strategies had an influence on the patients' OS and DFS.

3.7.1 Therapy strategies

There were several options how patients could be treated. For reasons of clarity and comprehensibility, we pooled the therapy options into 4 groups for our analysis.

Firstly, those who received an operation only on the tumor site and those received an operation on the tumor site and a neck dissection are defined as group "OP".

Secondly, those receiving an operation and an adjuvant radiotherapy are combined with those who underwent an operation and adjuvant chemoradiotherapy, this group is called "OP+RT/CRT".

Thirdly, patient who were treated with radiotherapy alone and those who received a concurrent chemotherapy were combined to the group "RT/CRT".

The fourth group consists of patient who had no therapy at all.

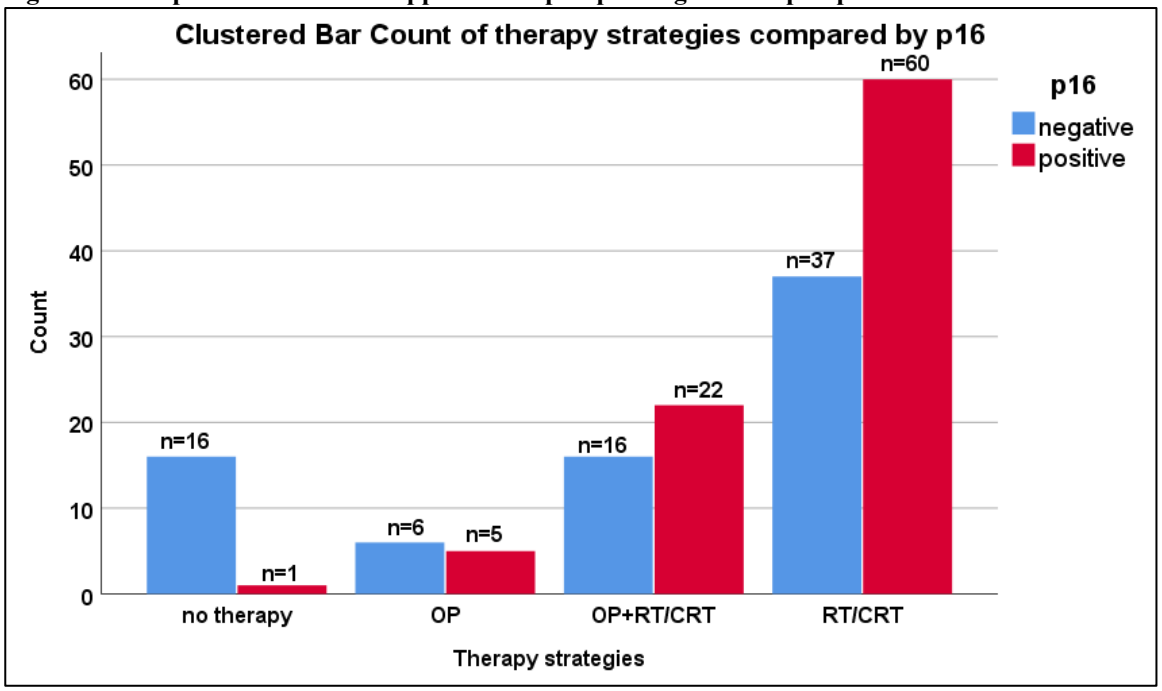
3.7.1.1 p16-negative OPSCC

In the p16-negative subgroup consisting of 79 patients 20,3% (n=16) patients received no therapy, 7,6% (n=6) surgical therapy, another 20,3% (n=16) patients received adjuvant therapy in combination with their surgery and 46,8% (n=39) were treated with primary RT/RCT, as outlined in Figure 32. 5,1% (n=4) received another form of treatment (e.g., a chemotherapy without radiotherapy) and were not included in the following analyses as we wanted to focus on the common paths of treatment.

3.7.1.2 p16-positive OPSCC

In the p16-positive subgroup consisting of 88 patients 1,1% (n=1) received no therapy, 5,7% (n=5) surgical therapy, another 25% (n=22) patients received adjuvant therapy in combination OP and 68,2% (n=60) were treated with primary RT/RCT, as outlined in Figure 32.

Figure 32 Comparison of count of applied therapies p16-negative vs. p16-positive OPSCC



This clustered bar chart shows the absolute frequency of applied therapies of p16-negative (blue) and p16-positive patients (red). Abbreviations: OP: operation, RT: radiotherapy, CRT: chemoradiotherapy

3.7.2 Therapy strategies-Overall survival functions

3.7.2.1 p16-negative OPSCC

For the overall survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all therapy strategies. The comparison between no therapy and OP+RT/CRT (p<0,001) and between no therapy and primary RT/CRT (p<0,001) showed a statistically significant difference. As shown in Figure 33a patients who received no therapy compared to patients who received surgery showed a lower OS, but without statistical significance (p=0,055). The same applies to the difference between patients with OP+RT/CRT and primary RT/CRT (p=0,052). The 1-year OS-rate for patients without any therapy was 23%, the 3-year OS-survival rate for patients who were treated with a surgical therapy was 83%, for those receiving adjuvant RT/CRT in addition to surgery it was 76% and for those were treated with primary RT/CRT it was 51%. Patients without any therapy had the shortest mean OS with 7,3 months (95% CI: 3,3-11,3), followed by patients with RT/CRT with 28,3 months (95% CI: 23,1-33,5), patients treated with surgery had a mean OS of 35,2 months (95% CI: 23,0-47,4) and patients with adjuvant CRT following their surgery of 36,9 months (95% CI: 31,4-42,4).

3.7.2.2 p16-positive OPSCC

For the overall survival in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all therapy strategies. Only the comparison between no therapy and surgery (p=0,025), no therapy and OP+RT/CRT (p<0,001) and between no therapy and RT/CRT (p<0,001) led to a statistically different result. There was no significant difference between patients with OP and OP+RT/CRT (p=0,450) or between patients with OP+RCT and primary RT/CRT (p=0,501). These three therapy strategies showed similar curves in Figure 33b. The 3-year OS-survival rate for patients who were treated with a surgical therapy was 100%, for those receiving adjuvant RT/CRT in addition to surgery it was 89% and for those were treated with primary RT/CRT it was 82%.

Figure 33 OS by therapy strategy a) p16-negative OPSCC b) p16-positive OPSCC

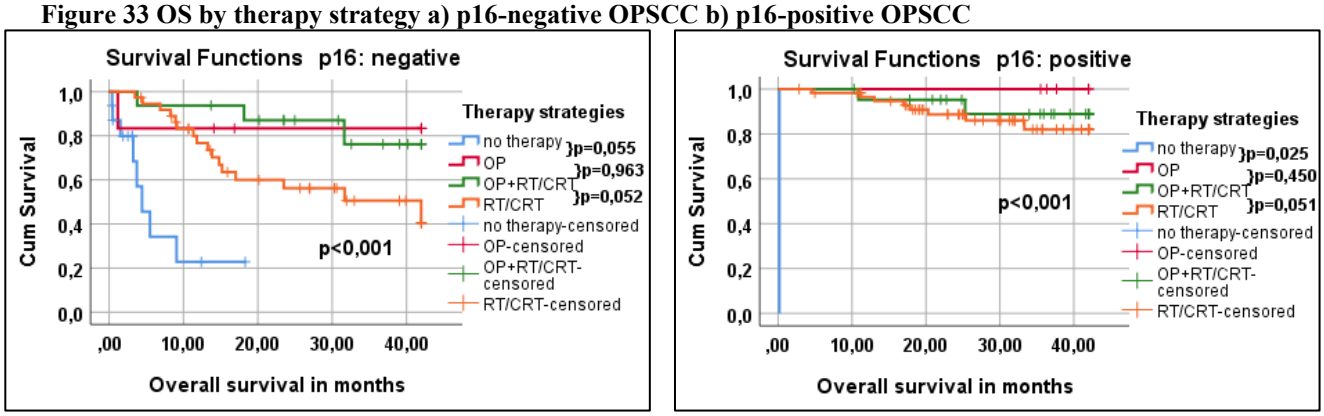


Figure 33a **Figure 33b**

These Kaplan-Meyer survival functions depict the differences in overall survival between the various therapy strategy groups of p16-negative (left, Figure 33a) and p16-positive OPSCC (right, Figure 33b). (Abbreviations: OP: operation, RT: radiotherapy, CRT: chemoradiotherapy)

3.7.3 Therapy strategies- Disease-free survival functions

3.7.3.1 p16-negative OPSCC

For the disease-free survival in p16-negative OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all therapy strategies. Only the comparison between no therapy and the other therapy strategies led to a statistically different result in this small cohort. (No therapy compared compared to OP (p=0,040) and OP+RT/CRT (p<0,001) and primary RT/CRT (p<0,001)). As shown in Figure 34a, patients who received RT/CRT compared to those who received OP+RT/CRT showed a lower DFS, but without statistical significance (p=0,064). There was no significant difference between patients with OP and OP+RT/CRT (p=0,441).

The 1-year DFS-rate for patient without any therapy was 11%, the 3-year survival rate for patients who were treated with a surgical therapy was 28%, for those receiving adjuvant RT/CRT in addition to surgery it was 51% and for those were treated with RT/CRT it was 32%. Patients without any therapy had the shortest mean DFS with 5,8 months (95% CI: 2.7-9,0), followed by patients with RT/CRT with 21,7 months (95% CI: 16,4-26,9), patients treated with surgery had a mean DFS of 23,9 months (95% CI: 11,5-36,4) and patients with adjuvant CRT following their surgery of 31,0 months (95% CI: 24,5-37,5).

3.7.3.2 p16-positive OPSCC

For the DFS in p16-positive OPSCC, Kaplan Meyer showed a statistically significant difference (Log Rank, p<0,001) among all therapy strategies. Only the comparison between no therapy and the other therapy strategies led to a statistically different result in this small cohort. (No therapy compared to OP (p=0,025) and OP+RT/CRT (p<0,001) and RT/CRT (p<0,001)). There was a difference but without significance between patients with OP and OP+RT/CRT (p=0,374) or between patients with OP+RT/CRT and primary RT/CRT (p=0,055), as seen in Figure 34b. Neither the 1-year DFS-rate nor the 3-year DFS-rate could be calculated for patient with no therapy. The 3-year survival rate for patients who were treated with a surgical therapy was 80%, for those receiving adjuvant RT/CRT in addition to surgery it was 85% and for those were treated with primary RT/CRT it was 62%. Patients with no therapy had the shortest mean DFS with 0,2 month (95% CI: 0,2-0,2), followed by patients with primary RT/CRT with 32,1 months (95% CI: 28,6-35,7). Patients treated surgically had a mean DFS of 38,9 months (95% CI: 33,9-43,9) and patients with adjuvant CRT following their OP of 37,99 months (95% CI: 33,7-42,3).

Figure 34 DFS by therapy strategy a) p16-negative OPSCC b) p16-positive OPSCC

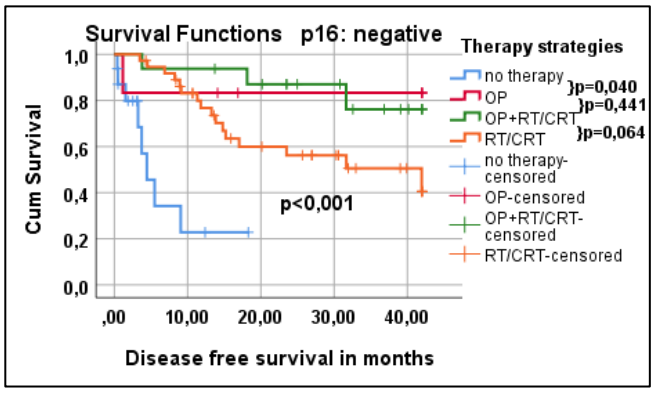


Figure 34a

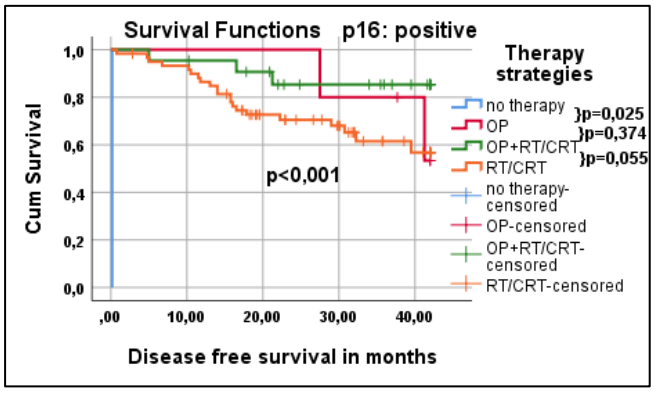


Figure 34b

These Kaplan-Meyer survival functions depict the differences in disease-free survival between the various therapy strategy groups of p16-negative (left, Figure 34a) and p16-positive OPSCC (right, Figure 34b). (Abbreviations: OP: operation, RT: radiotherapy, CRT: chemoradiotherapy).

3.7.4 Difference of therapy strategy decisions in p16-positive OPSCC before 2018 compared to after 2018

This chapter should shed some light upon the question if therapy strategies differ for p16-positive OPSCC when comparing patients before and after the implementation of the 8th Edition of UICC TNM classification (1). For this analysis p16-positive OPSCC were clustered in two groups, patients before 2018 and after 2018. It should be noted that for this calculation patients of both groups were classified according to the 8th Edition of UICC TNM classification (1) to be able to compare those two groups correctly. In addition, patients were pooled as early-stage (I and II) and advanced-stage (III and IV) disease.

3.7.4.1 Early-stage disease

For this analysis 58 patients with p16-positive OPSCC were considered, of which 48 were treated before 2018 and 10 after 2018. The results showed that before 2018 patients with p16-positive early-stage disease were treated with a surgical approach without adjuvant therapy in 10,4%, with surgery combined with adjuvant RT/CRT in 37,5% and with primary RT/CRT in 52,1%.

After 2018 patients with p16-positive early-stage disease were treated with surgery combined with adjuvant RT/CRT in 30,0 %, with conservative RT/CRT in 70,0% and no patients was treated with surgery only.

The differences in therapy strategy decisions between the group before 2018 and after 2018 were not statistically significant (Chi-Square, p=0,438).

3.7.4.2 Advanced-stage disease

For this analysis 30 patients with p16-positive OPSCC were considered, of which 22 were treated before 2018 and 8 after 2018. The results showed that before 2018 patients with p16-positive advanced-stage disease were treated with surgery combined with adjuvant RT/CRT in 4,5 % and with primary RT/CRT in 90,9% and one patient (4,5%) received no therapy.

After 2018 patients with p16-positive advanced-stage disease were treated with conservative RT/CRT in 100,0%.

The differences in therapy strategy decisions between the group before 2018 and after 2018 were not statistically significant (Chi-Square, p=0,677).

Figure 35 Comparison of therapy choices before vs. after 2018 a) early-stage disease b) advanced-stage disease

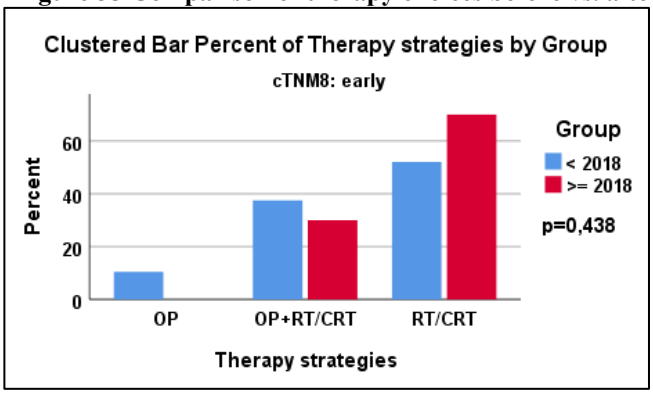


Figure 35a

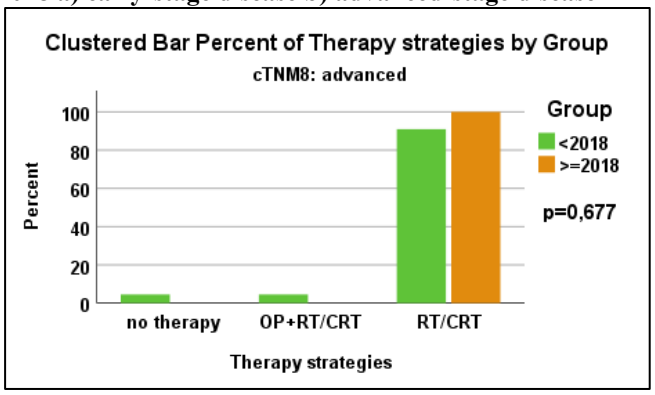


Figure 35b

Left, Figure 35a: This bar chart depicts the difference in chosen therapy strategies comparing patients with p16-positive early-stage disease (stage I and II) before 2018 (blue) to after 2018 (red).
Right, Figure 35b: This bar chart depicts the difference in chosen therapy strategies comparing patients with p16-positive advanced-stage disease (stage III and IV) before 2018 (green) to after 2018 (orange).
Abbreviations: OP: operation, RT: radiotherapy, CRT: chemoradiotherapy.

3.7.5 Influence of therapy on result of 1st Restaging

Whereas OS and DFS depict the outcome in a long-term way, the effectiveness of therapy can also be estimated with the result of the first restaging, as described in chapter 3.5.5.

We compared the results at the first restaging for p16-positive patients before 2018 to the results after 2018, both for the group of with early-stage disease and with advanced-stage disease. However, it must be noted that these described results are not directly dependent from the therapy strategy, only an indirect influence can be suspected. Therefore, we performed another analysis on p16-positive patients who were treated before 2018 trying to evaluate the impact of surgery on the result of the first restaging. This might indicate trends on which therapy strategy favors a positive result at the first restaging.

3.7.5.1 Early-stage disease

The results of the first restaging showed that before 2018 patients with p16-positive early-stage disease went into complete remission in 77,1%, another 12,5% were classified with PR, 2,1% with SD, 2,1% with PD and in 6,3% of the patients there was not adequate record of the first restaging. After 2018 patients with p16-positive early-stage disease were classified as CR in 100%. The differences in the results of the first restaging between the group before 2018 and after 2018 were not statistically significant (Chi-Square, p=0,587).

Figure 36b shows that patients with p16-positive early-stage disease have a high rate (>70%) of CR, both in patients who were treated surgically and those who were not. Among the patients who presented themselves with PR, SD, or PD at the first restaging, around two thirds were not operated and one third was treated surgically without adjuvant therapy.

Figure 36 Early-stage disease: Comparison of result of 1st restaging a) before and after 2018 b) tumor operated yes or no

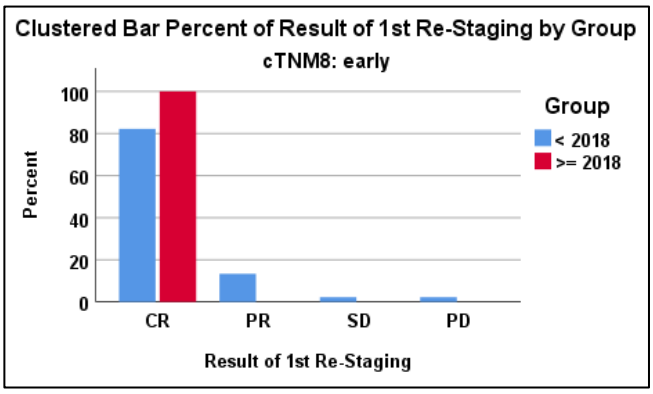


Figure 36a

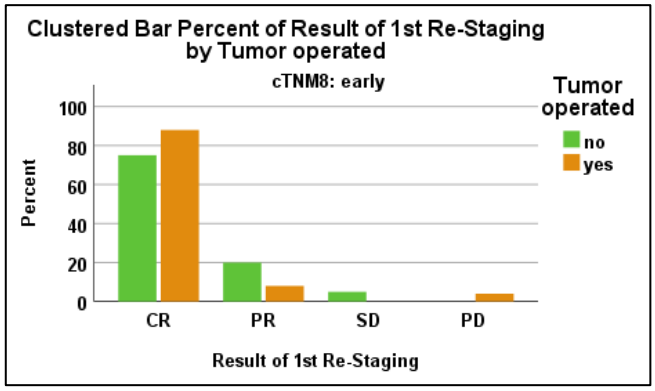


Figure 36b

Left, Figure 36a: This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive early-stage disease (stage I and II) before 2018 (blue) to after 2018 (red).
Right, Figure 36b**:** This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive early-stage disease (stage I and II) who were not operated (green) to those who were operated (orange).
Abbreviations: CR: complete remission, PR: part remission, SD: steady disease, PD: progressive disease

3.7.5.2 Advanced-stage disease

The results of the first restaging showed that before 2018 patients with p16-positive advanced-stage disease went into complete remission in 63,6%, another 18,2% were classified with PR, and 9,1% with SD. After 2018 patients with p16-positive advanced-stage disease went into complete remission in 50,0%, another 12,5 % were classified with PR, and 12,5 % with PD. The differences in the results of the first restaging between the group before 2018 and after 2018 were not statistically significant (Chi-Square, p=0,299).

Surgically treated patients with p16-positive advanced-stage disease presented with complete remission in 100% at the time of first restaging (referring to Figure 37b). In contrast, those who were not treated surgically were in complete remission in around 65% only.

Figure 37 Advanced-stage disease: Comparison of result of 1st restaging a) before and after 2018 b) tumor operated yes or no

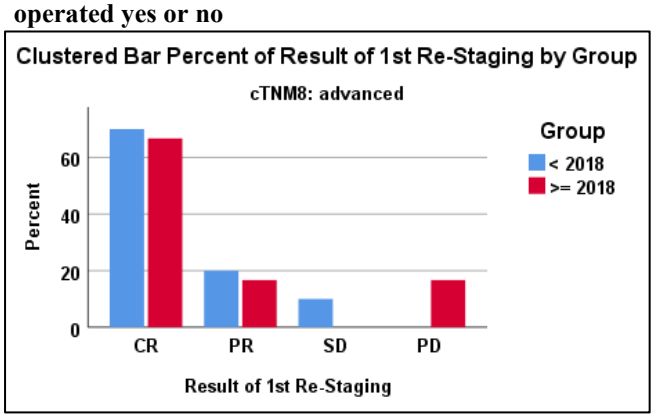


Figure 37a

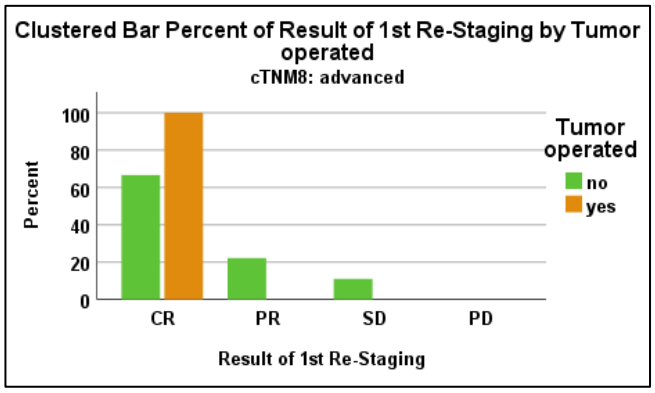


Figure 37b

Left, Figure 37a: This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive advanced-stage disease (stage III and IV) before 2018 (blue) to after 2018 (red).
Right, Figure 37b: This bar chart depicts the differences of the results of the first restaging comparing patients with p16-positive advanced-stage disease (stage III and IV) who were not operated on (green) to those who were operated on (orange).
Abbreviations: CR: complete remission, PR: part remission, SD: steady disease, PD: progressive disease

4 Discussion

4.1 Characteristics of our study collective

4.1.1 Prevalence of HPV

Several studies are investigating the prevalence of HPV in oropharyngeal cancer. As briefly described in chapter 1.2.4 Mehanna et al. (13) did a review and meta-analysis on the prevalence of HPV in OPSCC and focused on geographical and temporal differences. According to the study, the overall prevalence of HPV positive OPSCC is *'47,7% (95% CI: 42,9-52,5)*' ((13) p.747).

In Europe, the overall prevalence is *'39,9% (95% CI: 32,8-47)*' ((13) p.750). However, there could be identified statistically significant changes over time, the prevalence has risen from 32,2% in studies published before 2000 to 73,1% in studies published after 2005 ($p<0,004$). In contrast, in Northern America, studies showed that before 2000 HPV prevalence in patients with OPSCC was 50,7%, and after 2005 69,7% ($p<0,002$).

Compared to the study of Mehanna et al. (13), our results showed a lower prevalence than the alleged count in Europe after 2005. With 49,4% p16-positive OPSCC patients in our study cohort, the prevalence resembles the overall prevalence that Mehanna et al. indicate. As we do not have detailed data about the status of HPV positivity in OPSCC years back, we cannot state a rise in prevalence. Further studies in years to come could prove if the prevalence rises in Styria as well.

In addition, Mehanna et al. (13) claimed that the rise is of particular interest as a high prevalence in HPV-associated OPSCC influences the discussion of the cost-effectiveness of the HPV vaccine.

4.1.2 Tumor localization

When comparing the distribution of the tumor location in both groups, one can see that the tonsils and the base of the tongue make up the biggest part of the tumor locations as outlined in Figure 8. In addition, in both groups, tumors were equally at both sides of the pharynx. However, there were some differences between the two subgroups. Firstly, it seemed like the tumor in the p16-positive subgroup were more focused on the tonsils, which would align with the theory of Lyford-Pike et al. (11) that the tonsils are a privileged location for OPSCC due to its histology, as described in chapter 1.2.3. In contrast, in the p16-negative subgroup, the tumor was widely distributed in the oropharynx and could even appear in multiple sites. Secondly, no p16-positive tumors arised in the soft palatal arch in our patient cohort; only the p16-negative OPSCC did.

4.1.3 Age and gender

Patients who suffer from HPV-associated OPSCC are known to be younger than patients with HPV-negative tumors. According to Chaturvedi et al. (43) the mean age of HPV positive OPSCC was 61,0 years and for HPV-negative tumors 63,8 years ($p<0,001$). Interestingly our patients showed a contradicting result to published literature. In our analysis, the mean age of diagnosis was higher in patients with p16-positive OPSCC than in p16-negative ones (66,1 vs. 62,1 years, $p=0,010$). It is unclear why these results deviate from the common understanding in literature; maybe further investigation could shed some light on this topic.

El-Mofty et al. claimed that the distribution of HPV-positive OPSCC between gender showed a ratio of 4:1 in favor of men (44). However, the analysis of our p16-positive subgroup revealed a ratio of 3:1 men to women, and this would indicate that in our study cohort more women were suffering from p16-positive OPSCC than it is commonly known.

According to current literature, there are several hypotheses as to why men tend to suffer from p16-positive OPSCC more often than women. Firstly, men are more likely to have more sexual partners than women (45). Secondly, it is suspected that men are more likely to receive an oral HPV infection than women through oral sex, '*possibly because of higher vaginal/cervical HPV copy numbers.*' ((46) p. 2220). Thirdly, '*it has also been hypothesized that women develop a better immune response than men, after a corresponding genital HPV infection, and are thereby more resistant to developing an OPSCC.*'((47) p.141)

4.1.4 ECOG

The ECOG (Eastern Cooperative Oncology Group) performance score is a handy tool that estimates the restrictions a patient endures due to his tumor disease and is therefore of great interest to physicians in charge. Interestingly the HPV status also influences the patient's performance. According to Fakhry et al. (24) HPV-positive patients are more likely to perform better than their HPV-negative counterparts. Our analysis can sustain this hypothesis; we obtained very similar results compared to Fakhry et al. (24). Within the group of p16-positive OPSCC patients scored an ECOG 0 in 67% in our study vs. 66% in the American study (24) and an ECOG 1-2 in 30,7% (Styria) vs. 34% (USA). For p16-negative OPSCC our results showed an ECOG 0 in 39,2% vs. 38% (USA) and ECOG 1-2 in 50,7% (Styria) vs. 62% (USA). We could prove that the number of patients with an ECOG >1 was higher in the p16-negative subgroup, and in this group, there was also one patient with ECOG 4, as seen in Figure 9. Furthermore, we could report that the difference of ECOG

scores at initial presentation between p16-negative and p16-positive OPSCC was statistically significant in our patient collective (Mann-Whitney Test p<0,001).

Said results mean that most patients with p16-positive OPSCC did not have any daily life restrictions at initial presentation. In contrast, for p16-negative OPSCC patients, this was true for a much smaller portion of patients. However, they usually presented themselves with physical restrictions and additional diagnoses. Interestingly, p16-positive OPSCC are said to have earlier lymph node metastases than patients with p16-negative OPSCC (25) so even though the disease is earlier locally advanced than in HPV negative OPSCC, the disease itself affects the patient´s physical performance less.

4.2 Difference in survival between p16-positive and negative OPSCC

The published literature has a common consensus that the p16-status respectively HPV status contributes to a significant difference in survival for patients with OPSCC.

The results of this study can affirm this hypothesis. Our Kaplan-Meier survival functions could show that the overall survival (OS) rate and the disease-free survival (DFS) rate differ in patients regarding HPV positive versus HPV negative OPSCC, as described in chapter 3.3.1.4 and 0.

Compared to the US-American study of Ang et al. published in 2010 (23) we can report very similar results, especially regarding OS.

For p16-positive OPSCC, our analysis led to a 3-year OS rate of 84,6% compared to 83,6% in the US-American study (23), in DFS we could report a 3-year DFS rate of 68,1% compared to 74,4% in the US.

For p16-negative OPSCC our results showed a 3-year OS-rate of 54,4% compared to 51,3% in the US (23), and in DFS a 3-year DFS-rate of 33,5% (Styria) compared to 38,4% (USA).

Not only retrospective studies indicate the difference, but also several prospective studies such as the one performed by Fakhry et al. in 2008 (24). Their analysis could also '*provide prospective evidence that tumor HPV status is an independent prognostic factor for survival*' (p.268).

To sum up, consisting to existing literature, we can confirm the importance of treating p16-positive and p16-negative OPSCC as two different entities as p16-positive OPSCC has a better prognosis.

4.3 Adequacy of the 8th Edition UICC-TNM staging for p16-positive OPSCC

To begin with, for p16-positive OPSCC, the 8th Edition UICC TNM classification (1) underwent major changes for disease staging. When applying the new 8th version, many patients are classified with a lower stage compared to the previous 7th UICC TNM classification (27). As described thoroughly in chapter 3.4, we could prove that the patients' stages underwent a noteworthy shift. For example, in our analysis, when using the 7th Edition (27) the predominant stage was stage IVA whereas when applying the 8th Edition (1) the prevalent stage was stage I. In other words, p16-positive OPSCC patients were staged as an early-stage disease (stage I or II) in 12,5% using the 7th Edition vs. in 65,9% using the 8th Ed and as advanced-stage disease (stage III or IV) in 87,5% (7th Edition) vs. 34,1% (8th Edition) Other studies also reflected that shift in stage ((25),(39)). Moreover, according to van Gysen et al. (25) the predominant shift was from 7th Edition stage IV to 8th Edition stage I in 41,2%. To sum up, the new classification led to a considerable shift in stage for p16-positive OPSCC, as many of the patients were down-staged. We calculated the tumor stadium differences between the 7th and 8th Edition of UICC TNM classification for both p16-negative and p16-positive patients, and the results showed that 48,8% of patients in Styria were up- or down-staged at least one stage. This number was higher when only evaluating the p16-positive subgroup, in this case around 94% were down-staged at least one stage.

After the 8th UICC-TNM classification (1) was published, studies worldwide have been conducted to validate the new system. The main question of these studies was if the changes described above concerning the patient's tumor stage represented an accurate prognosis. Therefore, one of the primary ambitions of this thesis was to find out if the data of patients in Styria can endorse a benefit of the new TNM classification.

4.3.1 Overall survival

Our data shows, regarding the overall survival of p16-negative OPSCC patients, both the 7th (27) and the 8th (1) TNM versions enable physicians to give a proper prognosis as both versions reflect the outcome in a reasonable way. The classification for p16-negative OPSCC did not change very much (changes are described in chapter 1.4.2.2). However, in the p16-positive group the classification changed a lot and for the better, as our data indicate. In the graph showing the overall survival when applying the new 8th Edition of the UICC TNM staging system (Figure 16a), stages were in the expected order, unlike in the diagram for the 7th Edition (Figure 16b), which indicates that staging of the 8th TNM Edition can make more reliable and more accurate predictions. However, it should be noted that we cannot say this

as a fact, as our analyses did not lead to statistically significant differences in overall survival and disease-free survival between the individual stages.

Nevertheless, we also analyzed the overall survival when clustering the patients in early-stage disease (stage I and II) and advanced-stage disease (stage III and IV). Interestingly the analyses regarding p16-positive OPSCC showed that when applying the 7th Edition (27) there was no significant difference between the early-stage and advanced-stage disease (p=0,685; Figure 17a), which changes with the utilization of the new 8th Ed (1) (p=0,012; Figure 17b). Moreover, using the 7th Edition (27) resulted in a better overall survival (OS) in patients with advanced-stage disease compared to early-stage disease, paradoxically. Application of the 8th Edition of TNM corrected this misrepresentation. Now, patients with advanced disease could anticipate a worse OS than those with early-stage disease, as one would expect.

As mentioned above, several studies aiming to validate the new 8th UICC TNM classification (1); Würdemann et al. (Germany, 2017, (39)) claimed that the 8th Edition (1) better discriminated between the stages in p16-positive OPSCC and was a better prognostic tool. However, they could only prove a statistically significant difference in OS between stage I-II and III-IV, not between II and III. The same applies to the Australian study conducted by van Gysen et al. (2019, (25)), which also confirmed that in comparison to the previous 7th Edition (27) , the 8th Edition allowed a more accurate prognosis. Still, van Gysen et al. (25) reported a lack of significant difference between OS in stages II and III. Interestingly the Japanese study of Mizumachi et al. (2017, (40)) resulted in a statistically significant difference in OS between stage II and III, albeit there was no difference between stage I and II.

4.3.2 Disease-free survival

When analyzing the disease-free survival (DFS) of our study cohort, conclusions are not that clear. For the p16-negative subgroup, the Kaplan Meyer survival functions showed hardly any significant differences between the individual stages. The curve of Stage IVC, meaning patients with distant metastases, showed lower disease-free survival than the other stages. But apart from that stage, the comparison did not show apparent differences graphically (Referring to Figure 18a and b). Furthermore, patients with stage III cancer showed the longest DFS, even longer than those with stage I or II, both when applying the 7th (27) and 8th (1) of the UICC TNM classification. These findings indicate that for p16-negative OPSCC the TNM classification is a useful prognostic tool for OS, but for DFS, prognoses are not that trustworthy.

Interestingly, our analysis proved that for p16-positive OPSCC the 8th Edition of UICC TNM classification (1) was a slight improvement to predict DFS. The analysis of the p16-positive subgroup, classified with the 7th Edition (27), showed that patients with disease stage III might have the longest mean DFS, as seen in Figure 19a. Implementing the 8th TNM Edition (1) eliminated this unrepresentative observation (Figure 19b) by downstaging stage III patients. Patients with stage I tumors had the best DFS, followed by stage II, then stage III, and lastly stage IV. However, the analysis of p16-positive OPSCC proved that the only stage with significant differences was stage IVC (7th Edition) respectively IV (8th Edition). These results are supported by Figure 19a and Figure 19b, showing a reduced disease-free survival in stage IV, respectively IVC.

When analyzing the disease-free survival of patients with p16-positive OPSCC by clustering them in early-stage (stage I and II) and advanced-stage disease (stage III and IV), the difference was not statistically significant when applying the 7th Edition (27) (p=0,632; Figure 20a). This changes with the usage of the 8th Edition of UICC TNM classification (p=0,025; Figure 20b). Moreover, one can observe better discrimination between the early-stage and advanced-stage disease graphically.

De Felice et al. (UK, 2020, (48)) could also demonstrate in their study cohort that p16-positive patients benefit from the 8th Edition of UICC TNM classification (1) as it allowed to give more accurate prognoses when it comes to terms of disease-free survival. In addition, they could prove, similarly to our analysis, that there was better discrimination between the stages.

4.3.3 What could lead to a better prognosis?

However, a common consensus in the published literature seems to be that the 8th Edition UICC TNM classification (1) is an improvement compared to its predecessor regarding the predictive value for p16-positive OPSCC. Regardless, they all claim that it still needs refinement as it differs inadequately between certain stages. (25, 39)

Therefore, we tried to determine which aspect of the TNM classification would improve and did an additional analysis of OS and DFS regarding separated T categories, respectively separated N categories.

Our analysis of the T categories showed that for p16-negative OPSCC the Kaplan Meyer overall survival functions discriminated between the individual T categories graphically as seen in Figure 21a and b. Still, these results could not be supported by statistical significance. On the other hand, for p16-positive OPSCC, our analysis showed that the overall survival was similar for all T categories when applying the 8th Edition of UICC TNM staging (1).

Neither a graphically obvious discrimination between the individual stages (Referring to Figure 22a and b), nor statistically significant differences in OS or DFS could be demonstrated. In addition, we also performed an analysis regarding the influence of the T category on the results of the first restaging in p16-positive OPSCC. The results did not lead to remarkable new findings, as T1, T2, and T3 tumors showed comparable results and only T4 tumors had a worse performance at the first restaging. Several other studies prove these facts, that distinction in outcome between the smaller T categories is not clear; only the category with the most considerable extent of primary tumors (T4) shows a difference.

The analysis of the N category in p16-negative OPSCC did not lead to new results. It did not show statistical differences between the individual N categories concerning overall survival or disease-free survival. The analysis of p16-positive OPSCC demonstrated that the N categories of the 8th Edition of TNM are an improvement compared to its predecessor. Overall survival analyses using the 8th Edition of UICC TNM classification showed significant differences in OS between N2 and N3 (p=0,002), as seen in Figure 26b. The analysis of disease-free survival resulted in a significant difference between N1 and N2 (p=0,047) and between N2 and N3 (p=0,006), as seen in Figure 28b. In contrast, applying the 7th Edition of UICC TNM classification, there were no significant differences between individual N categories, neither regarding OS nor DFS.

Moreover, we also investigated the influence of lymph node metastasis (N category) on the result of the first restaging. This analysis showed that early-stage disease patients with N2 were in CR in 50% and in PR in 50%. In addition, all patients with advanced-stage disease and N3 presented exclusively with PR at the first restaging. These findings indicate once again that high extent lymph node metastasis leads to a worse outcome and highlight the potential of the N category as a predictive tool.

To sum up, the results of our study could indicate that when the TNM classification gets further improved in the future, the main focus for p16-positive OPSCC research should be the nodal spread and less the primary tumor size.

In contrast, to deepen the precision of the TNM classification, another thinkable approach could be to stop classifying OPSCC in many stages and start using the terms early- and advanced-stage disease. Figure 15b, depicting the overall survival functions of p16-negative OPSCC when applying the 8th Edition of UICC TNM classification (1), might create the impression of two main groups; Stages I, II, and III showed similar survival functions and so did stage IVA, IVB and IVC. For p16-positive OPSCC, when staged according to the 8th Edition of UICC TNM classification (1) this formation of two groups was even more evident,

as seen in Figure 16b. In addition, our analyses of OS (Figure 17b) and DFS (Figure 20b) led to statistically significant differences and a graphically clear distinction between early- and advanced-stage disease.

In the future, the discovery and establishment of biomarkers can help to improve the reliability of prognoses for oropharyngeal carcinoma.

4.3.4 Implementation of the 8th Edition UICC TNM in the Head and Neck tumor board

One part of our analysis was evaluating the process of implementing the new 8th Edition of UICC TNM classification (1) as thoroughly described in chapter 3.6. The staging of patients in 2018 performed by the physicians of the Head and Neck tumor board was confirmed by the reviewing study team, who corrected the initial staging if needed, affecting only a few cases. However, it should be noted that in some cases the p16-status was not yet determined at the time of the initial staging in the Head and Neck tumor board. Further, the study team classified patients retrospectively, having complete patient information present, which could explain some of these differences and slight deviations. Moreover, in the group staged in 2018, there were no significant differences between official and retrospective TNM staging. All in all, our analysis showed that the implementation of the 8th Edition of UICC TNM classification (1) did not cause major adjustment difficulties and went smoothly. This indicates that the new rules of classification are understandable and easily applicable.

4.4 Influence of therapy decision on survival

As mentioned above, the p16-status dramatically influences the outcome of the patients as p16-positive patients tend to have better overall survival and disease-free survival. Another relevant aspect for clinical practice is if the p16-status also affects the success of therapy. Therefore, we analyzed both OS and DFS of p16-positive and p16-negative OPSCC comparing the individual therapy strategies (no therapy vs. OP vs. OP+RT/CRT vs. RT/CRT as described in chapter 3.7.1).

For p16-negative OPSCC our analysis showed that, apart from patients without therapy, comparing therapy strategies, no statistically significant differences in OS and DSF could be found. However, as shown in Figure 33a, patients with p16-negative OPSCC who received primary chemoradiotherapy have lower overall survival than those who received surgery, with or without adjuvant therapy; the same goes for disease-free survival (Referring to Figure 34a).

Figure 33b depicting the OS of p16-positive OPSCC can create the impression that both conservative and surgical therapy approaches can lead to good survival for patients as

graphically differences between therapies are not that obvious. Statistically, there is no significant difference between OP+RT/CRT and RT/CRT (p=0,051). On the other hand, when analyzing the disease-free survival, our Kaplan-Meier survival functions (Figure 34b) show that p16-positive OPSCC with a surgical approach have better disease-free survival than those receiving RT/CRT.

All in all, our data suggest that both p16-positive and p16-negative OPSCC patients benefit from surgery compared to a therapy regimen without a surgical approach only using radiotherapy or chemoradiotherapy. However, the reduced success of RT/RCT compared with surgery and RT/RCT is less distinct in p16-positive OPSCC than in p16-negative OPSCC. This might support the hypothesis that p16-positive OPSCC patients show a better response to RT/RCT, '*reflecting higher intrinsic sensitivity to radiation or better radiosensitization with the use of cisplatin.'* ((23) p.34)

4.4.1 Changes in therapy decisions after 2018

In addition, we also wanted to analyze whether therapy strategies changed for p16-positive OPSCC with the implementation of the 8th Edition of UICC TNM staging. Therefore, we analyzed therapy decisions before and after 2018; patients were pooled in early-stage and advanced-stage disease. Furthermore, we also evaluated the results of the first restaging in those groups, which indicates the success and early outcome of the therapy.

The analysis of the early-stage patients showed that after 2018 a rise in the number of conservative therapies could be observed (>=2018: 70% vs. <2018: 52,1); however, this difference is not significant (p=0,438). Patients were classified with complete remission in 100% after 2018 and over 80% before 2018. In addition, we analyzed the early-stage patients before 2018 more thoroughly and we could show that among those who were not in CR, around two-thirds were primarily treated with primary chemoradiotherapy, and one-third were treated surgically.

The analysis of the advanced-stage disease showed that in the group after 2018, 100% of the patients were treated with a primary conservative approach vs. around 91% in the group before 2018. In both groups, patients showed a similar rate of CR (>65%). However, more thoroughly analyzing advanced-stage patients before 2018, the results showed that those treated primary surgically showed a CR of 100%. Among those who primary received conservative therapy, the CR rate was 65%.

To sum up, our data indicates a trend that after 2018 p16-positive patients tend to be treated more often with primary conservative therapy. However, in both early- and advanced-stage disease, patients with a surgical approach tend to have better results at the first restaging.

4.4.2 Should p16 status have an impact on therapy decisions?

The theory of higher response of p16-positive OPSCC to conservative therapy leads to many discussions which therapy modality is the best for p16-positive OPSCC and if it should differ to p16-negative OPSCC. Up to date, this question has not been utterly answered and is controversially discussed.

Wang et al. (41) published a systematic review in 2015 analyzing therapy strategies for both p16-negative and p16-positive OPSCC. Their results showed that p16-negative had a worse outcome when treated with chemoradiotherapy, suggesting that surgical approaches should be favored if feasible. For p16-positive OPSCC results were not that clear, the authors claim that primary surgery led to better survival than primary chemoradiotherapy; however, hazard ratios were not significantly different (41).

In contrast, Lybak et al. (2017) claim that, regarding their population in Norway, patients with p16-positive OPSCC did not have better survival when treated with primary surgery than with primary chemoradiotherapy, but a worse quality of life and therefore recommend a conservative regimen. Surgical approaches included in this study were, among others, also extensive procedures such as free flap reconstructions. (49)

However, primary chemoradiotherapy versus surgery and chemoradiotherapy is not the only question currently investigated, but also the potential of dosage de-escalation for patients with p16-positive OPSCC. Dosage de-escalation could be a tremendous benefit for patients; less toxicity and possibly fewer long-term consequences might result in a better quality of life (41). Chera et al. (USA, 2018) conducted a study with de-intensified radiation (60 Gy vs. 70 Gy) and chemotherapy (Cisplatin 180 mg/m2 vs. 300 mg/m2) for patients with a tumor extent of T0-3, N0-N2c (TNM7) respectively N0-N2 (TNM8), M0. They could report an overall survival rate of 95% and less burden of symptoms and improved quality of life (50). Misiukiewicz et al. (51) also support the hypothesis of therapy de-escalation in their study of 2019 (USA), claiming that patients who received dosage-reduced radiotherapy following induction chemotherapy showed a comparable survival to patients receiving the standard of care dosage while reporting higher quality of life. (51)

To sum up, various clinical trials are investigating the best therapy for p16-positive OPSCC, of which results are still inconsistent. Thus, the National Comprehensive Cancer Network (NCCN) does not recommend a p16-status-specific treatment in their guidelines published in 2018 (30). Of note, the most crucial influence on therapy decisions should remain the individual traits of the patients, such as tumor extent, nodal extension, and the patient's general condition.

4.5 Is HPV-positive OPSCC preventable?

There are different types of prevention. The term "primary prevention" means that measures are taken to prevent the disease in the first place. "Secondary prevention," means that, the disease is detected in an early-stage and therefore curative therapy course is possible.

4.5.1 Primary prevention

4.5.1.1 Abstinence and barrier protection

As mentioned before, oro-genital sex is the leading behavioral risk factor for receiving an oral HPV infection (52). Furthermore, the amount of lifetime sexual partners contributes also to this risk (45). Therefore, one way of preventing OPSCC would be to not engage in oro-genital sex and a promiscuous lifestyle. As this might not always be feasible, another approach would be using barrier protection. Gupta et al. (53) claim that using barrier protection such as condoms and dental dams reduces the risk of an oral HPV infection evidently (*'RR: 0.21; 95% CI: 0.04–0.97; $p < 0.05$'* (53) p. 612) in unvaccinated individuals. Thus, the authors emphasize the general need to educate adolescents about the danger of HPV infections and the importance of using protection even during oro-genital intercourse. The authors explicitly state that this is especially of utmost importance for an individual without a vaccination respectively those who are not eligible for a vaccination. (53)

4.5.1.2 Vaccination

Vaccine

The role of HPV as an oncogenic factor in the development of cervical cancer has been known for over 20 years (54), whereas the knowledge about the influence of the virus on OPSCC is a younger one. Therefore, the initial intent of developing a vaccine against HPV was to prevent cervical cancers. After thorough research, the quadrivalent vaccination GARDASIL ® Merck & Co., Inc (55) was approved in 2006 (46) protecting against the HPV types 6, 11, 16, and 18. This vaccination proved to be an effective method to prevent HPV infections and was refined. Today GARDASIL 9 ® (Merck Sharp & Dohme Corp.) is available on the market and this nonavalent agent also protects against the HPV types 31, 33, 45, 52, and 58 additionally to the mentioned four HPV types of GARDASIL 4 (56). With the growing evidence of HPV-associated oropharyngeal cancers, several studies examined the vaccine's influence on oral HPV infections. For instance, Chaturvedi et al. proved in their research in 2018 that in the US the "*prevalence of vaccine-type oral HPV infections (HPV16/18/6/11) was significantly reduced in vaccinated versus unvaccinated individuals*

18 to 33 years of age" ((57) p. 263). The U.S. Food and Drug Administration listed the prevention of oropharyngeal and other head and neck cancers in men and women aged 9 to 45 as one of the official indications of GARDASIL 9 since August 2020 (56).

Situation in Austria

In Austria, the vaccination against HPV is part of the official schedule for immunization and prevention of infectious diseases (58). The nonavalent HPV vaccine GARDASIL 9 is highly recommended, free of charge for girls and boys between 9 and 12 years, and is usually part of school-based vaccination programs in Austria. As the vaccination is most effective when applied before children reach a sexual-active age, this costless program should motivate parents to get their children vaccinated early. Additionally, until the age of 15, the treatment can be obtained at a reduced price. The vaccination schedule states that the immunization is implicitly recommended until the age of 30; later, the application is optional. Furthermore, the authors of the vaccination schedule explicitly state the importance of vaccinating all genders to disrupt the chain of infection and to achieve herd immunity. In Austria it is advised to administer two shots of the GARDASIL 9 vaccine, the second shot within six to twelve months after the first one. (58)

Global situation

Globally, several countries strive for a high vaccination prevalence against HPV in young girls and boys. In Europe, apart from Austria, eight other countries, namely Italy, Germany, Liechtenstein, Switzerland, Norway, the United Kingdom, Croatia, and the Czech Republic, have or plan a vaccination program addressing both young girls and boys (59). Australia should be highlighted with a free prevention program which started in 2007 for girls and included boys after 2013. A study published by Brotherton et al. in 2017 showed that in 2015 around 78% of 15-year-old girls received all three shots of the quadrivalent HPV vaccine and 67% of the 15-year-old boys (60).

Expected decline in OPSCC burden

As mentioned above in chapter 4.1.3 the median age of diagnosis in HPV positive OPSCC is 61 years (43). Since it takes some time until vaccinated adolescents reach the average age of disease onset of 60-65 years, results cannot be expected soon. Gillson et al. anticipate that the incidence trends of oropharyngeal carcinomas will reverse after 2060. However, they specifically remark that this prediction assumes that the vaccine's provided protection is long-lasting. (61)

The role of the HPV vaccine in the prevention of disease recurrence in patients whose p16-positive OPSCC was successfully treated has to be determined in the future.

4.5.2 Secondary prevention

To start with, the main problem in the secondary prevention of oropharyngeal carcinomas is the absence of precursor lesions, unlike cervical cancer, where the Papanicolaou test enables physicians to detect and treat precancerous lesions (52). Therefore, researchers aim to find different testing methods to detect OPSCC earlier.

Firstly, one approach was to test the saliva and search for the presence of HPV DNA. However, even though the method could detect the active virus, its diagnostic value is limited as the "*majority of individuals either go on to clear the infection or fail to progress to malignancy*" ((52) p.1923). Therefore, this method is not used as a screening method for HPV.

Secondly, Kreimer et al. (62) demonstrated in their study from 2017 that antibodies to the E6 oncogene of HPV16 could be a marker for the development of oropharyngeal cancer. The antibodies were positive and stable in patients for many years prior to the diagnosis of OPSCC and the authors estimate '*the 10-year absolute risk to be 6.2% for HPV16-E6-seropositive men*' ((62) p.6). However, the authors state that further investigation is needed, specifically when establishing the following steps after an E6-seropositive result. It is unknown in which intervals patients should be clinically examined and receive thorough imaging. (62).

Thirdly, transcervical sonography could detect cervical masses early. As lymph node swelling can have many reasons apart from carcinomas, the diagnostical value is limited. However, as it is cheap, conducted quickly, and has no danger of ionizing radiation, the method can be considered (52).

Fourthly, there are approaches in refining and using imaging methods to detect cancerous mucosal lesions of the oropharynx. For example, the technology of narrow-band imaging displaying vascular tissue better is beneficial for detecting tumors that are usually highly vascularized. Lifetime-resolved laser-induced imaging is another imaging method; when the laser is applied, the reflected signals differ whether the laser reaches healthy tissue or cancerous lesions (52).

Fifthly, one exciting approach which will be more thoroughly investigated in the future is the concept of circulating cell-free DNA, also known as liquid biopsies. When tissue dies, the cell´s DNA is released into the blood and circulates for several minutes. Malign tumors have a fast turnover of cells, leading to a high load of DNA being shed into the serum, which then can be detected. HPV-positive OPSCC patients often present with prominent nodal metastases, often with substantial necrosis, benefiting this detection method. One big perk

of this method is that it is micro-invasive and still can give a lot of information about the tumor (52).

To sum up, there are several promising approaches in detecting oropharyngeal carcinomas early. Still, more investigation and research must be done until these methods are well established in the clinical routine. However, the main focus in preventing HPV-associated cancers of the oropharynx should be the primary prevention and vaccinating young people.

4.6 Limitations to our study

There are several limitations to our study. To begin with, it is a retrospective data analysis including limitations that are universally true for this kind of study. For instance, the study cohort was rather heterogeneous and probably not a balanced sample. Possible confounders were not evaluated, and additional information that might be interesting for analysis cannot always be collected retrospectively.

In addition, in some of our cases, medical records were incomplete; for example, imaging workup data was partially not found in MEDOCS or not even performed in a minimal part of the cases. Moreover, some patients disrupted their follow-up appointments, and these limited data could also cause bias.

Another limitation to our study is the restricted number of patients who were included in our study. Of note, oropharyngeal squamous cell carcinomas are not to be found very frequently compared to other malignant tumors, so that limits the number of patients per se. In addition, our initial attempt was to include patients diagnosed and treated in 2013 and 2014 too, but it turned out that p16-status was not adequately documented in various cases, leading to limited valid information for the said period. On the other hand, there would not be much use in including patients after 2018, due to a short duration of follow-up.

4.7 Conclusion

With the upcoming rise of HPV-induced oropharyngeal squamous cell carcinomas (OPSCC) (13), it is of utmost importance to know the details of this kind of carcinomas.

The analysis of data of a tertiary care center in Styria revealed that from 2015 to 2018 49,4% of all OPSCC were p16-positive. This number conforms with the overall prevalence of 47,7%, which is claimed by Mehanna et al. (13). In our study cohort, the mean age at diagnosis was lower for p16-negative patients (62,1 years) than for p16-positive patients (66,1 years); this difference of means was statistically significant (p=0,010). These results contradict common results in published literature, where patients with p16-positive OPSCC got diagnosed at a younger age (43). Also, in our study cohort, women made up 25% of all

p16-positive OPSCC; this percentage was slightly higher compared to published literature, as El-Mofty et al. claim that around 20% of p16-positive OPSCC were women. (44). Our analysis could prove that patients with p16-positive OPSCC have a better ECOG score at initial presentation which means that they had better performance and lesser restrictions in daily life than patients with p16-negative OPSCC; this difference was statistically significant ($p<0,001$). Further, we proved a superior survival of p16-positive OPSCC compared to p16-negative ones. Not only did p16-positive OPSCC have a better overall survival (OS) but also a better disease-free (DFS)-survival.

The new 8th Edition of UICC TNM classification (1) released in 2017 treats p16-positive OPSCC as a different entity and therefore has different staging rules than for p16-negative OPSCC. These new rules led to a notable shift in staging. Our results showed, corresponding to published literature, that many patients who would have been classified as an advanced-stage disease with the 7th Edition of UICC TNM classification (27) were now classified with lower stages when applying the 8th Edition. As these adapted TNM classification rules needed to be reviewed, several studies evaluated their validity (25, 39, 40). Our analysis also aimed to prove the superiority of the new 8th Edition of UICC TNM classification (1). When analyzing the OS of p16-positive patients, the Kaplan-Meier-survival functions showed that the 8th Edition has better reliability of prognosis graphically, as the survival functions of the individual stages were in the correct order as outlined in Figure 16b. However, apart from the difference between stage III and IV ($p<0,001$), our results could not rely on statistically significant differences. The same coud be said when analyzing DFS. When grouping patients in early-stage (stage I and II) and advanced-stage (III and IV), our analysis could prove that when applying the 8th Edition of UICC TNM classification, the OS functions better discriminate between the two groups, and this difference was statistically significant ($p=0,012$). Again, the analysis of DFS led to similar results; early and advanced-stage disease also differed significantly ($p=0,025$) in DFS.

All in all, we could demonstrate that the 8th Edition of UICC TNM classification is an improvement, corresponding to published literature (25, 39, 40). Nevertheless, these authors claim that there is still a lack of clarification between individual stages. Therefore, we evaluated OS and DFS of p16-positive OPSCC when stratifying them by individual T-categories or N-categories, respectively. Our results showed no evident difference in OS between the individual T categories as seen in Figure 22. Better DFS of T1 tumors compared to the other T categories is outlined in Figure 24. However, again the tumor extension seemed to have only a minor impact on survival. The analysis of the individual N categories

showed that the 8th Ed (1) led to more precise information on overall survival functions than the 7th Ed (27); however, the only significant difference could be found between N2 and N3. When analyzing the DFS of p16-positive OPSCC, there can also be found a considerable difference between N1 and N2, and again between N2 and N3. Therefore, we would propose that, if future refinement of the TNM classification needs to be done, the revision should be focused on the N categories, less the primary tumor extension.

In addition, we also wanted to evaluate the implementation process of the 8th Edition of UICC TNM classification (1) and we can report that it went smoothly and without major adjustment difficulties.

It is still an ongoing discussion whether p16-positive OPSCC should be treated differently than p16-negative ones, we also evaluated OS and DFS, stratifying by therapy strategies. For p16-negative OPSCC, we could demonstrate that radiotherapy/chemoradiotherapy (RT/CRT) led to a worse survival than surgical therapy approaches. The evaluation of p16-positive OPSCC showed that both OP and RT/CRT led to good results in OS; however, the DFS is better for patients who received an operation. Of note, the survival functions of p16-positive OPSCC treated with RT/CRT were less inferior than in the p16-negative subgroup, which would align with the hypothesis that p16-positive OPSCC patients show a better response to conservative treatment (23). In addition, our analysis also indicates that the p16-positive OPSCC OP group had a higher rate of complete remission than those with primary RT/CRT. To sum up, in both subgroups, our results indicate that patients benefit from surgical treatment if feasible. However, to answer the question of the best cure for p16-positive OPSCC, more thorough prospective research must be conducted in the future.

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Figure 5 Histological depiction of p16+OPSCC and Figure 6 Detection methods for HPV can be used as a courtesy of Zhong Chen, MD, PhD et al.; these figures were copied from the paper “Current status of clinical testing for human papillomavirus in oropharyngeal squamous cell carcinoma” primarily published in 2018 (15).

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