Diplomarbeit

Oropharyngeal squamous cell carcinoma: Evaluation of pre-therapeutic Plasma Fibrinogen and C-reactive Protein as prognostic factors

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Adrian Knoflach

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unter der Anleitung von

Ass. Dr. med. univ. Prisca Pondorfer

Univ. Prof. Dr. med. univ. Dietmar Thurnher

Graz, am 1.8.2018

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Graz, am 1.8.2018 *Adrian Knoflach eh*

I. Danksagung

An dieser Stelle möchte ich mich bei meinen Eltern bedanken, die mir dieses Studium ermöglicht haben.

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II. Zusammenfassung

Seit geraumer Zeit ist bekannt, dass Entzündung eine Schlüsselrolle in der Entstehung von Krebs spielt. Verschiedene entzündungsspezifische Biomarker werden in einigen Krebsentitäten als prognostisches Mittel verwendet. Wenige Studien zeigen einen Zusammenhang von Biomarkern, wie beispielsweise CRP, und Oropharynxkarzinomen. Das Ziel dieser Studie ist die Evaluation von CRP und Fibrinogen als mögliche prognostische Faktoren beim Oropharynxkarzinom.

In dieser retrospektiven Studie wurden Daten von 161 Patientinnen und Patienten der HNO des LKH-Graz analysiert und Parameter mittels Kaplan-Meier-Schätzer und Cox Regression auf deren Signifikanz überprüft. Primäre Endpunkte waren das Gesamtüberleben und das krankheitsfreie Überleben während eines Beobachtungszeitraumes von 31 ± 23.2 Monaten.

T-Klassifikation, AJCC-Stadium, ECOG-Stadium und CRP-Erhöhung zeigten eine statistisch signifikante Korrelation. Erhöhte CRP-Werte waren signifikant assoziiert mit schlechterem Gesamtüberleben in der gesamten Kohorte (p=0,003), der Oropharynx-Gruppe (p = 0,023) und in der Zungengrund-Gruppe (p = 0,029), sowie mit schlechterem krankheitsfreien Überleben in der gesamten Kohorte (p<0,001), der Oropharynx-Gruppe (p=0,003), der Tonsillen-Gruppe (p =0,011) und der Zungengrund-Gruppe (p =0,039). Fibrinogen zeigte keine Signifikanz im Gesamtüberleben oder Krankheits-freiem Überleben.
Mit dieser Studie können wir die Rolle von CRP als unabhängiger, prognostischer Marker der Oropharynxkarzinome bestätigen. Dieser Marker könnte in Zukunft als wertvolle Entscheidungshilfe in der Therapieplanung gelten.

Schlüsselwörter: Oropharynxkarzinom, CRP, Fibrinogen, Gesamtüberleben, krankheitsfreies Überleben

III. Abstract

It has been known for a long time that inflammation plays a key role in the genesis of cancer. Different biomarkers representing inflammation are being acknowledged and used as prognostic tools in various cancer types. Yet, there are only few studies published regarding biomarkers, such as CRP (C reactive protein), and oropharyngeal cancer. Thus, the aim of this work is to evaluate the potential of C-reactive protein and pretreatment plasma
fibrinogen as prognostic factors in oropharyngeal squamous cell carcinoma.

In this retrospective study charts of 161 patients at the department of Otorhinolaryngology of the University Hospital of Graz were reviewed and data was collected to investigate routine laboratory parameters (Fibrinogen and CRP) as prognostic factors. Kaplan-Meier method and Cox regression were applied for statistical analyses. Primary endpoints were overall survival (OS) and disease-free survival (DFS) during a mean follow-up of 31 ± 23.2 months.

T-classification, AJCC-stage, ECOG-stage and CRP showed a significant impact for the survival in general. Elevated levels of CRP were significantly associated with worse OS in the total cohort (p=0,003), the oropharynx group (p=0,023) and in the root-of-the-tongue group (p=0,029), as well as lower DFS in the total cohort (p<0,001), the oropharynx group (p=0,003), the tonsil group (p=0,011) and the root-of-the-tongue group (p =0,039). Levels of fibrinogen did not show any imact on OS or DFS.

In this study we could confirm the role of CRP as an independent prognostic marker in patients with oropharyngeal carcinoma. Presented biomarker CRP may serve as a liquid biopsy for prediction of OSCC especially for larger tumor sizes (T-classification), overall survival and disease free survial and improves decisions making for clinical approach.

Keywords: oropharyngeal cancer, CRP, fibrinogen, overall survival, disease-free survival

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V. Table of abbreviations

AJCC	American Joint Committee on Cancer
AUC	area under the curve
CRP	C-reactive protein
CSS	cancer-specific survival
DFS	disease-free survival
DMFS	distant-metastasis free survival
ECM	extracellular matrix
ECOG	Eastern Cooperative Oncology Group
GM-CSF	granulocyte-macrophage colony-stimulating factor
HPV	Human papillomavirus
IL-1	Interleukin-1
IL-6	Interleukin-6
MCP	monocyte chemotactic protein
OPC	oropharyngeal cancer
OOSCC	oral and oropharyngeal squamous cell cancer
OS	overall survival
OSCC/OPSCC	oral/oropharyngeal squamous cell carcinoma
Rb	Retinoblastoma protein
TAMs	tumor-associated macrophages
UICC	Union internationale contre le cancer
VEGF	Vascular Endothelial Growth Factor

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1. Introduction

1.1 Anatomy

1.1.1 The oral cavity

The oral cavity is the first part of the digestive tract and is divided by the upper and lower dental arches into the vestibule and the actual cavity, mouth cavity proper. The vestibule is located anteriorly and is the space between the lips/cheeks and the gums/teeth.
The mouth cavity proper, which lies posteriorly to the vestibule, is bordered by a roof, the hard and soft palate, the cheeks and a floor, which consists of the tongue, a muscular diaphragm, the geniohyoid muscles and the salivary glands and ducts.

1.1.1.1 Palate

The palate separates the oral cavity from the nasal cavity and is divided into two parts, the anterior hard palate, and the posterior, shorter soft palate. The hard palate consists of two bones, the palatine process of the maxilla, and the horizontal plate of the palatine bone. The bones are connected through the intermaxillary suture and the interpalatine suture.
The soft palate, also known as the velum, consists of five muscles and connective tissue, and does not contain bone. The muscles are the m. levator veli palatine, tensor veli palatine, m. palatopharyngeus, m. palatoglossus and the m. uvulae.

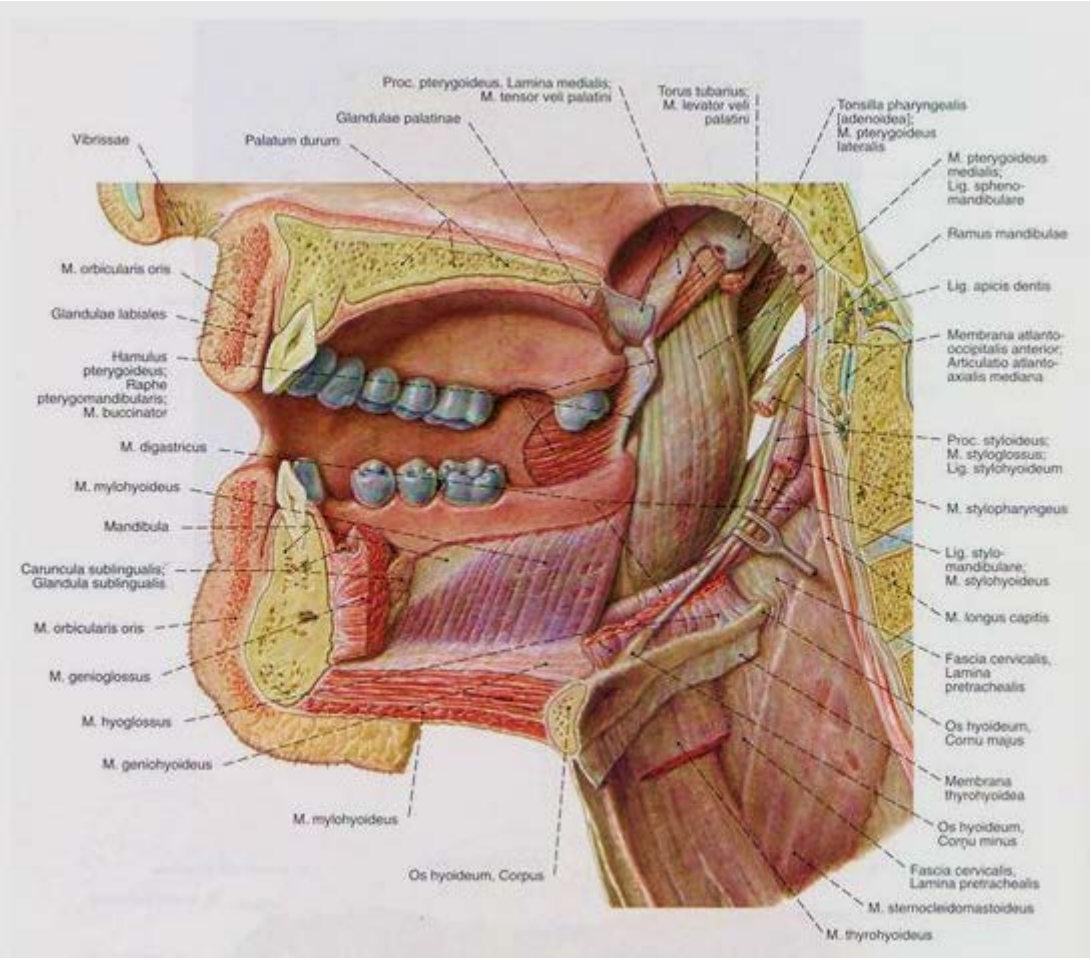


Figure 1: The oral cavity.[1]

A sagittal view of the oral cavity and its structures.

1.1.2 Tongue

The tongue is divided into two parts, the lingual corpus and the lingual radix. The eight muscles of the tongue are classified as either extrinsic, which are attached to bone, or intrinsic, which are not attached to any bone. The extrinsic muscles are the m. genioglossus, m. styloglossus, m. hyoglossus and m. palatoglossus. The intrinsic muscles are the superior longitudinal muscle, the inferior longitudinal muscle, the vertical muscle and the transverse muscle.

1.1.2.1 Blood supply of the tongue

The tongue and the floor of the mouth both receive blood supply from the lingual artery, a branch of the external carotid artery. The lingual and sublingual vein, drain into the internal jugular vein via the facial vein.

1.1.2.2 Nerve supply of the tongue's mucosa

The nerve supply of the tongues mucosa consists of several nerves:

- Sensation: the lingual nerve, a branch of the mandibular nerve, innervates the anterior two thirds of the tongue, the glossopharyngeal nerve innervates the posterior third, and the superior laryngeal nerve, a branch of the vagus nerve, innervates the base of the tongue.
- Taste: the chorda tympani branch of the facial nerve innervates the fungiform papillae, the glossopharyngeal nerve innervates the vallate and foliate papillae, and the vagus nerve the base of the tongue.

1.1.2.3 Nerve supply of the tongue's muscles

The hypoglossal nerve supplies all extrinsic and intrinsic muscles.

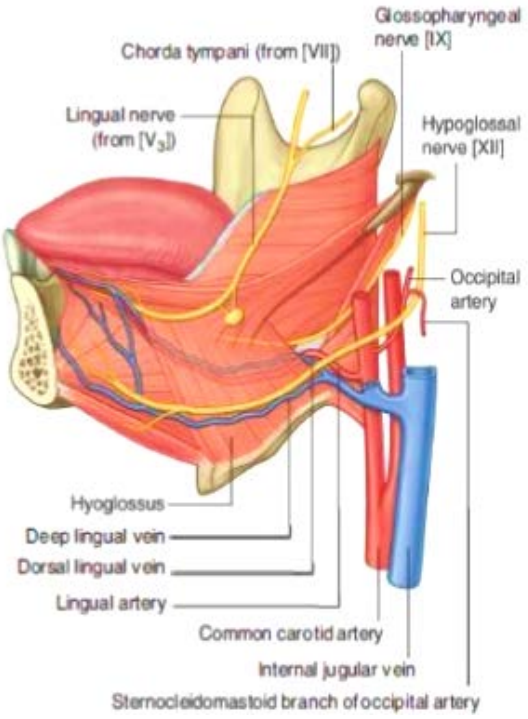


Figure 2: Nerve supply of the tongue's muscles.

All muscles are innervated by the hypoglossal nerve [XII]. The lingual artery, a branch of the external carotid artery, supplies the tongue and the floor of the mouth.[2]

1.1.3 Pharynx

The pharynx is part of the digestive system and the respiratory system. It also contains lymphoid tissue, known as the Waldeyer's lymphatic ring. The pharynx extends from the base of the skull to the sixth cervical vertebra and is divided into three sections, the nasopharynx, the oropharynx and the laryngopharynx (= hypopharynx).
The nasopharynx extends from the base of the skull to the upper surface of the soft palatine and communicates anteriorly with the nasal cavities through the choanae.[3]
The oropharynx is the middle part of the pharynx and is located between the soft palate superiorly, where it communicates with the nasopharynx, and the top of the epiglottis inferiorly, where it communicates with the hypopharynx. Anteriorly it is continuous with the oral cavity and posterior is the pharyngeal wall.[4]
The caudal part of the pharynx, the laryngopharynx, is the largest part. It is located inferior to the epiglottis and connects to the esophagus.

1.1.3.1 Blood and nerve supply of the pharynx

Arteries:
The external carotid artery supplies the ascending pharyngeal artery, and the ascending palatine artery, which is a branch of the facial artery. Moreover, the laryngopharynx is supplied by the inferior thyrocervical artery, a branch of the thyrocervical trunk, which is supplied by the subclavian artery.
Veins:
Venous drainage is achieved by the pharyngeal and pterygoid plexus, which drain into the jugular vein.
The innervation of the pharynx is achieved by the pharyngeal plexus, which consists of branches of the glossopharyngeal nerve, branches of the vagus nerve and the sympathetic trunk.

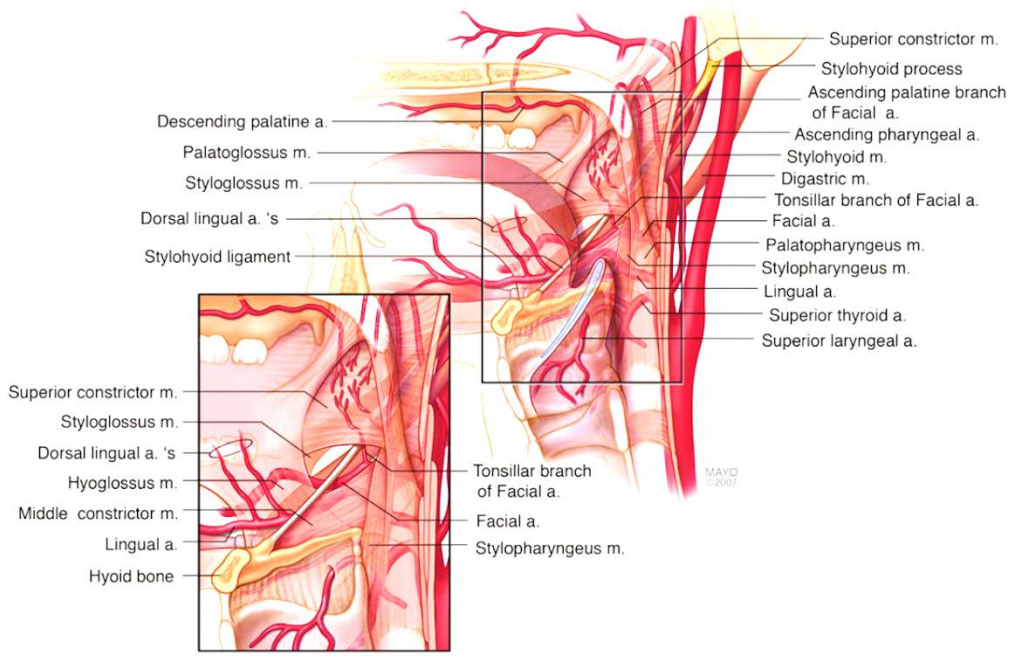


Figure 3: Blood supply of the pharynx.

The pharynx receives blood from the ascending pharyngeal artery and the ascending palatine artery, which are both supplied by the external carotid artery. The laryngopharynx is supplied by the inferior thyrocervical artery.[5]

1.1.4 Tonsils

The Waldeyer's lymphatic ring consists of the pharyngeal tonsil, tubal tonsil, lingual tonsil, palatine tonsil and mucosa associated lymphatic tissue in the saplingopharyngeal fold.

1.1.4.1 Blood and nerve supply of the tonsils

The ascending pharyngeal artery supplies pharyngeal branches, the lingual artery supplies lingual dorsal branches and the facial artery supplies the ascending palatine artery, which supplies tonsil branches. The innervation is achieved by the glossopharyngeal nerve and the maxillary nerve.[6]

1.1.5 Cervical lymph nodes

Lymph nodes in the neck have been divided into seven levels, generally for the purpose of squamous cell carcinoma staging as outlined in Figure 4. In addition, there

are other several important groups, such as the supraclavicular, parotid, retropharyngeal space, and occipital nodes.

Level I

Margins of cervical lymph node level I:

- above the lower margin of the hyoid bone
- anterior to the posterior border of the submandibular glands
    - Level Ia: submental nodes located between the anterior part of the left and right digastric muscle
    - Level Ib: submandibular nodes located posterolateral to the anterior part of the digastric muscles and anterior to the posterior border of the submandibular glands

Level II

The cervical lymph nodes of level II are part of the internal jugular chain, also known as the deep cervical group.

- cranial to the caudal margin: base of the skull to the inferior border of hyoid bone
- anterior margin: posterior border of sternocleidomastoid muscle (SCM)
- posterior margin: posterior border of the submandibular glands
    - level IIa: anterior, lateral, medial, or posterior to the internal jugular vein. If the node is posterior to the internal jugular vein, it must be inseparable from it.
        - most superior node is the jugulodigastric node
    - level IIb: posterior to the internal jugular vein with fat layer separating the nodes and the vein

Level III

The cervical lymph nodes of level III are also part of the internal jugular chain.

- cranial to the caudal margin; lower border of hyoid to lower border of cricoid cartilage (or the intermediate tendon of omohyoid muscle)
- anterior margin: posterior border of SCM
- lateral margin: medial margin of the common carotid artery (CCA) / internal carotid artery (ICA)

Level IV

The cervical lymph nodes of level IV are the lower part of the internal jugular chain.

1) cranial to caudal margin: lower border of the cricoid cartilage to level of the clavicle
2) anterior margin: medial to an oblique line drawn through the posterior edge of SCM and the posterolateral edge of the anterior scalene muscle.
3) lateral margin: medial margin of the CCA

Level V

The cervical lymph nodes of level V are also called the posterior triangle (spinal accessory) nodes, located posterior to the back of the sternocleidomastoid muscle and anterior to the trapezius muscle.

- level Va: superior half, posterior to levels II and III, between base of the skull and inferior border of cricoid cartilage
- level Vb: inferior half, posterior to level IV, between the inferior border of cricoid cartilage and the level of clavicles

Level VI

The cervical lymph nodes of level VI are also called the prelaryngeal and pretracheal nodes or “Delphine” node.

- cranial to caudal margin: from the inferior border of hyoid bone to the manubrium
- posterior margin: visceral space
- lateral margin: anterior to cervical lymph node levels III and IV

Level VII

Superior mediastinal nodes are located between common carotid artery and below superior aspect of manubrium to level of the brachiocephalic vein[7]

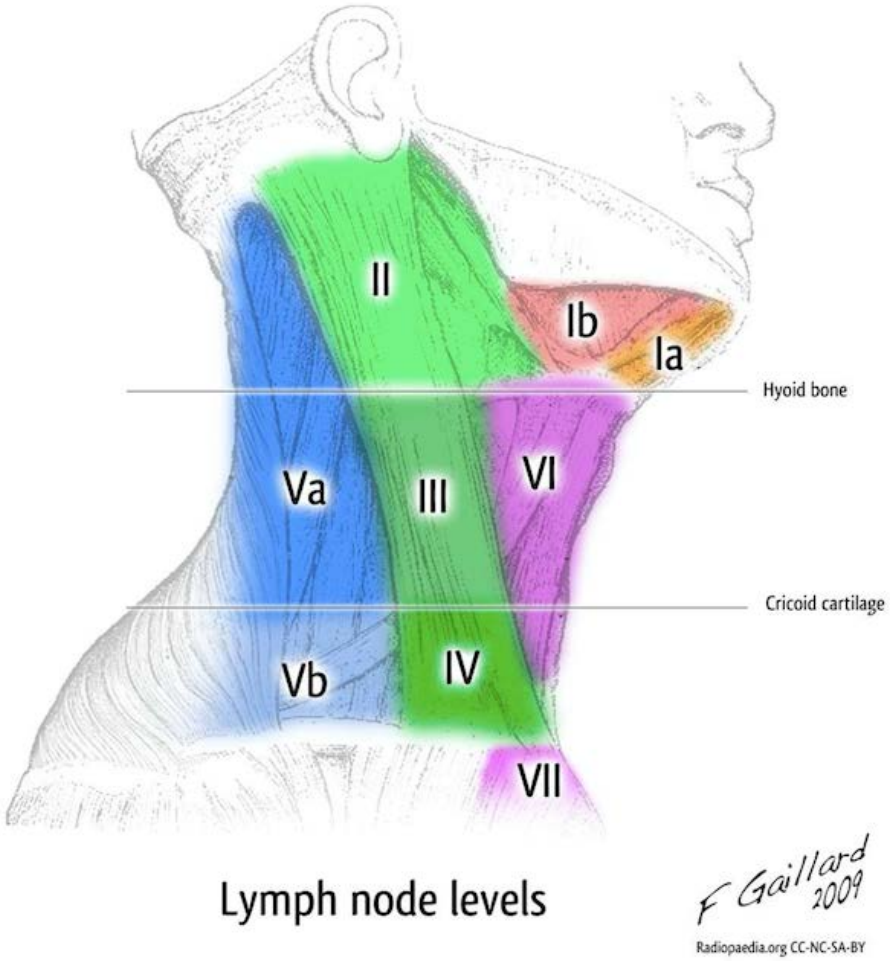


Figure 4: Lymph node levels

This figure shows levels of cervical lymph nodes I-VII. Main landmarks are the hyoid bone the cricoid cartilage and the sternocleidomastoid muscle.

Case courtesy of A. Prof Frank Gaillard, Radiopaedia.org, rID: 9618[8]

1.2 Epidemiology of OPSCC

Worldwide there are approximately 135000 cases of oropharyngeal cancer (OPC).[9]
The most common malign oral and oropharyngeal tumor is the squamous cell carcinoma, 3-4% of all malign tumors in Europe and USA are oral/oropharyngeal squamous cell carcinoma (OSCC/OPSCC). Typically, it occurs in the sixth through seventh decades of life and the incidence in men is 2 – 3 times higher than in women.[10]
In many parts of the world, there has been a significant increase of incidence of oropharyngeal squamous cell cancer (OPSCC).[11] Sweden for instance experienced a 3-fold increase between 1970 and 2002.[12]

1.3 Etiology of OPSCC

Well known etiological factors are alcohol and smoking: Smokers have a 2 – 4 times higher risk of developing OSCC/OPSCC, in smokers with additional abuse of alcohol the risk is 6 – 15 times higher, but alcohol has been found to be an independent risk factor as well.[13] Furthermore, Human papillomavirus (HPV) is recognized to be another risk factor of head and neck squamous cell carcinomas, with the strongest association in OPSCC. According to Carole Fakhry et al., HPV is observed in 40% – 60% of patients with OPSCC.[14] Particularly those arising in the base of the tongue and in the tonsillar region do not have the traditional risk factors.[15]
There are more differences in HPV-associated OPSCC compared with HPV-negative OPSCC: there is a strong preponderance of male gender in HPV-associated OPSCC[16]. Furthermore, Shao Hui Huang et al. described that patients with HPV-associated OPSCC are more likely to present with early-stage (T1/T2) primary tumor compared with HPV-negative patients and HPV-positive patients are less likely to develop a second malignancy[17].
Although the prevalence of HPV – negative cancer declined by 50% in the United States as a result of decreased incidence of smoking, HPV – positive OPSCC increased by 225%, according to the Surveillance, Epidemiology, and End Results (SEER) program's tissue repository data from 1988 to 2004, thus making OPSCC increasing in incidence. [18]

1.4 Tumor localizations of OPSCC

Localizations can be tonsils, root of the tongue including the posterior third of the soft palate (inferior surface or uvula), posterior pharyngeal wall and or the vallecula, the fold located between the base of the tongue and the epiglottis. The most common locations for primary tumors in oropharynx are tonsil and root of the tongue.

1.5 Staging of OPSCC

The TNM and AJCC classification is used to stage OPSCC. In December 2016, a new version of TNM classification, the AJCC 8th edition, was published which divides oropharyngeal carcinomas in two groups, p16 positive and p16 negative squamous cell carcinoma, whereas the p16 negative group is the same as the old classification. In this work, the AJCC 7th edition was used for staging and treatment of patients. Both classifications are outlined below:

AJCC 7th edition (older version) – classification of oropharyngeal cancer

Tumor size and margins (T):

- TX: primary tumor cannot be assessed
- T0: No primary tumor
- Tis: Carcinoma in situ
- T1: Tumor < 2cm in greatest dimension
- T2: Tumor >2cm but not more than 4cm in greatest dimension
- T3: Tumor >4cm in greatest dimension or extension to lingual surface of the epiglottis
- T4a: Moderately advanced, local disease: tumor invades the larynx, deep muscle of the tongue, medial pterygoid, hard palate, or mandible
- T4b: Very advanced, local disease: tumor invades lateral pterygoid muscle, pterygoid plates, lateral nasopharynx, or skull base or encases the carotid artery

Regional lymph nodes (N):

- NX: Regional nodes cannot be assessed
- N0: No evidence of regional lymph nodes
- N1: Metastasis in a single ipsilateral regional lymph node <3cm in greatest dimension
- N2: Metastasis in a single ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension; or in multiple ipsilateral/contralateral/bilateral lymph nodes, none >6cm in greatest dimension
- N2a: Metastasis in a single ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension
- N2b: Metastasis in multiple ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension
- N2c: Metastasis in bilateral/contralateral lymph nodes >3cm but not more than 6cm in greatest dimension
- N3: Metastasis in a lymph node > 6cm in greatest dimension

Distant metastasis (M):

- MX: Distant metastasis cannot be assessed
- M0: No distant metastasis
- M1: Distant metastasis

Staging AJCC 7th edition:

Stage 0:	Tis	N0	M0
Stage I:	T1	N0	M0
Stage II:	T2	N0	M0
Stage III:	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
Stage IVA:	T1-3	N2	M0
	T4a	N0-N2	M0
Stage IVB:	any T	N3	M0
	T4b	any N	M0
Stage IVC:	every T	every N	M1

AJCC 8th edition (new version) – changes in staging for regional lomphnodes(N)

Regional lymph node classification (N) for p16 (–) negative OPSCC (pathological and clinical)

N0: No evidence of regional lymph nodes

N1: Metastasis in a single ipsilateral regional lymph node <3cm in greatest dimension

N2: Metastasis in a single ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension; or in multiple ipsilateral/contralateral/bilateral lymph nodes, none >6cm in greatest dimension

N2a: Metastasis in a single ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension

N2b: Metastasis in multiple ipsilateral regional lymph node >3cm but not more than 6cm in greatest dimension

N2c: Metastasis in bilateral/contralateral lymph nodes >3cm but not more than 6cm in greatest dimension

N3: Metastasis in a lymph node > 6cm in greatest dimension

Staging AJCC 8th edition - for p16 (–) negative OPSCC:

Stage I:	T1	N0	M0
Stage II:	T2	N0	M0
Stage III:	T1	N1	M0
	T2	N1	M0
	T3	N0, N1	M0
Stage IVA:	T1-3	N2	M0
	T4a	N0-N2	M0
Stage IVB:	any T	N3	M0
	T4b	any N	M0
Stage IVC:	every T	every N	M1

Regional lymph node classification (N) for p16 (+) positive OPSCC

Clinical N-classification:

cN0:	no lymph nodes
cN1:	unilateral lymph node not more than 6cm
cN2:	contra- or bilateral lymph node not more than 6cm
cN3:	lymph node more than 6cm

AJCC – clinical staging:

Stage I:	T1,T2	N0,N1	M0
Stage II:	T1, T2	N2	M0
	T3	N0,N1,N2	M0
Stage III:	T1-T4	N3	M0
	T4	any N	M0
Stage IV:	any T	any N	M1

Pathological N-classification:

pN0:	no lymph nodes
pN1:	1 – 4 lymph nodes
pN2:	more than 5 lymph nodes

AJCC – pathological staging:

Stage I:	T1,T2	N0,N1	M0
Stage II:	T1, T2	N2	M0
	T3	N0,N1	M0
Stage III:	T3,T4	N2	M0
Stage IV:	any T	any N	M1[19]

1.6 ECOG – Scale of Performance Status

The ECOG (Eastern Cooperative Oncology Group) “Scale of Performance Status” is a measurement to evaluate how the disease impacts a patient’s daily living abilities. In 6 grades it describes a patient’s level of functioning in terms of their ability to care for themselves, daily activity, and physical activity (walking, working, etc.).

- Grade 0: Fully active, able to carry on all pre-disease performance without restriction
- Grade 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
- Grade 2: Ambulatory and capable of selfcare but unable to carry out any work activities; up and about more than 50% of waking hours.
- Grade 3: Capable of limited selfcare; confined to bed or chair more than 50% of waking hours.
- Grade 4: Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
- Grade 5: Dead[20]

1.7 Clinical presentation of patients with OPSCC

The clinical symptoms may vary with the location of the tumor, generally there can be pain, dysphagia, voice changes, weight loss, a mass in the neck and bleeding. Tonsillar lesions may also present with ipsilateral referred otalgia. If a tumor is located in the root of the tongue and infiltrates the deep muscle, the result can be a fixation of the tongue. Bleeding may occur with a tumor of the pharyngeal wall.[21],[22]

Metastases follow through the lymphatic vessels of the pharyngeal wall to the retropharyngeal and deep cervical lymph nodes,[23] distant metastases at initial diagnosis are normally asymptomatic; the most common sites are the lungs, the liver and bone[24].

It is interesting that HPV-positivity in the oropharynx patients represent a unique entity compared to HPV-negative. This distinction along with other clinical characteristics of each is summarized in the table below.[25]

Cancer site:	Oropharynx carcinoma	
	HPV neg.	HPV pos.
Common localizations:	• Pharyngeal wall • Soft palate	• Tonsils • Base of tongue
Common presentations:	• Sore throat • Dysphagia • otalgia	• Painless neck mass
Demographics:	• Smoker/ drinker • Older • Lower education	• Non-smoker • Male • Younger • Multiple partner • Higher education

Table 1: Clinical presentation

Common demographics and clinical characteristics of patients with oropharyngeal carcinoma. Abbreviations: HPV (Human papillomavirus)[26]

1.8 Diagnosis of OPSCC

Aside from the patient's anamnesis the diagnosis is made by inspection, palpation, if possible mirror examination, ultrasound, biopsy, computer tomography and PET – CT scan; the presence of a tumor must be confirmed histologically. Moreover, a metastatic work-up with appropriate imaging is recommended for all head and neck cancer patients, with particular attention to regional lymph node spread, lung spread and liver spread.

1.9 Treatment of OPSCC

The treatment includes a multimodality approach that may include surgery with ipsi- or bilateral regional resection of the cervical lymph nodes (neck dissection), chemotherapy, radiotherapy and immunotherapy. It depends on which stage the patient's carcinoma is:

T1:	surgical therapy
T2:	combination of surgery and radiotherapy
T3, T4:	with or without palliative tumor resection before primary radiotherapy/ chemotherapy/ immunotherapy [27]

After the patient is being discussed in the tumor board council, an individual treatment plan will be made. Dental evaluation is recommended prior to therapy, as well as nutrition, speech and swallowing evaluation. An audiogram is clinically indicated prior to chemotherapy containing platinum.

1.10 Surgical principles in OPSCC

Assessment of resectability

Tumor involvement of the following sites is associated with poor prognosis or function:

- Involvement of the pterygoid muscles, particularly when associated with severe trismus or pterygopalatine fossa involvement with cranial neuropathy
- Gross extension of the tumor to the skull base
- Direct extension to the superior nasopharynx or deep extension into the Eustachian tube and lateral nasopharyngeal walls;
- Invasion of the common or internal carotid artery. Encasement is usually assessed radiographically and defined as a tumor
- surrounding the carotid artery by 270 degrees or greater
- Direct extension of neck disease to involve the external skin
- Direct extension to mediastinal structures, prevertebral fascia, or cervical vertebrae
- Presence of subdermal metastases

None of these sites of involvement are absolute contraindications to resection in selected patients in whom total cancer removal is possible.[28]

Primary tumor resection

The resection of advanced tumors of the oropharynx will vary in extent depending on the structures involved. En-bloc resection of the primary tumor should be attempted whenever feasible. In-continuity neck dissection is necessary when there is direct extension of the primary tumor into the neck. As thickness of the lesion increases, the risk of regional metastases and the need for adjuvant elective neck dissection also increases. Perineural invasion should be suspected when tumors are adjacent to motor or sensory nerves. Transoral robotic or laser-assisted resections of primary cancers are increasingly used approaches for cancer resection in selected patients with limited disease and accessible tumors, not only in the oropharynx but also in the oral cavity, hypopharynx and larynx. Nevertheless, successful application of these techniques requires specialized skills and experience.[29]

Neck Management

Guidelines apply to the performance of neck dissections (ND) as part of treatment of the primary tumor. In general, patients undergoing surgery for resection of the primary tumor will also undergo dissection of the ipsilateral side of the neck, as this is the greatest risk for metastases.[30]

The type of neck dissection (comprehensive or selective) is determined at the discretion of the surgeon, and is based on the initial preoperative staging and tumor localization as follows:

<u>clinical staging: cN0</u>

Oral cavity:	at least levels I-III (selective ND)
Oropharynx:	at least levels II-IV (selective ND)
Hypopharynx:	at least levels II-IV and level VI when appropriate (selective ND)
Larynx:	at least levels II-IV and level VI when appropriate (selective ND)

<u>clinical staging: from cN1 to cN2a-c</u>

Selective or comprehensive neck dissection

<u>clinical staging: cN3</u>

Comprehensive neck dissection

1.11 Principle of chemotherapy in OPSCC

1.11.1 Cisplatin

The process of cell division, whether normal or cancerous cells, is through the cell cycle. The cell cycle goes from the resting phase, through active growing phases, and then to mitosis. Cisplatin is classified as an alkylating-like agent. Although cisplatin is frequently designated as an alkylating agent, it has no alkyl group and it therefore cannot carry out alkylating reactions. It is correctly classified as alkylating-like.[31] Alkylating and alkylating-like agents are most active in the resting phase of the cell. If the patient is not suitable for Cisplatin due to reduced general conditions, carboplatin is alternatively used.

1.11.2 Carboplatin

Carboplatin is less potent than cisplatin and it may only be 1/8 to 1/45 as effective. The clinical standard of dosage of carboplatin is usually a 4:1 ratio compared to cisplatin. The stable property of carboplatin has its advantages and disadvantages, once uptake of the drug occurs, its retention half-life is considerably longer than cisplatin, but it is also this inertness that causes carboplatin to go right through the human body, and a high percentage (up to 90%) of the carboplatin given can be detected in urine. In terms of its structure, carboplatin differs from cisplatin in that it has a bidentate dicarboxylate. Carboplatin exhibits lower reactivity and slower DNA binding kinetics, although it forms the same reaction products in vitro at equivalent doses with cisplatin.[32]

1.11.3 Side effects of chemotherapy in OPSCC

Cisplatin: In terms of onset, duration, and severity, Cisplatin side effects are often predictable. Common side effects (occurring in greater than 30%) for patients taking Cisplatin are nausea and vomiting, low blood counts resulting in an increased risk for infection, anemia, and/or bleeding, kidney toxicity, ototoxicity and blood test abnormalities like low magnesium, low calcium and low potassium. Less common side effects (occurring in 10-29%) for patients receiving cisplatin are peripheral neuropathy,

sensory loss, numbness and tingling, difficulty in walking, loss of appetite, taste changes and hair loss.[33]

Carboplatin: Common side effects (occurring in greater than 30%) for patients taking carboplatin are nausea and vomiting, low blood counts, taste changes, hair loss, weakness and blood test abnormalities like abnormal magnesium levels.
The less common (occurring in 10-29%) side effects for patients receiving carboplatin are burning sensation at the injection site, abdominal pain, constipation, diarrhea, mouth sores, peripheral neuropathy, sensory loss, numbness and tingling, difficulty in walking, central neurotoxicity, nephrotoxicity, ototoxicity, abnormal blood electrolyte levels (sodium, potassium, calcium) and abnormal blood liver enzymes.[34]

1.12 Principle of immunotherapy

1.12.1 Cetuximab

Cetuximab is an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of various cancers, such as metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer. Cetuximab is a chimeric monoclonal antibody. However, a randomized phase III clinical trial failed to show any improvement in survival with the addition of eight weeks of cetuximab to cisplatin and radiotherapy.[35] Recently, a biomarker of altered immune function, FCGR2A, was found to influence the response rate to cetuximab for patients with Kirsten rat sarcoma viral oncogene homolog (KRAS)-mutant tumors, indicating that inherited polymorphisms affect cetuximab response.[36],[37]

1.12.2 Other immunotherapy agents

Other immunotherapy agents are also available for OPSCC after detailed clinicopathological evaluation of the patient and its tumor:

- Nivolumab: It is a human IgG4 anti-PD-1 monoclonal antibody and works as a checkpoint inhibitor, blocking a signal that would have prevented activated T-cells from attacking the cancer, thus allowing the immune system to clear the cancer.[38]

- Pembrolizumab: It is an IgG4 isotype antibody that blocks a protective mechanism of cancer cells, and allows the immune system to destroy those cancer cells. It targets the programmed cell death 1 (PD-1) receptor of lymphocytes.[39]

1.12.3 Side effects of immunotherapy with cetuximab

Grade	1	2	3	4	5
Hypersensitivity (allergic reaction)	Transient flushing or rash; drug fever <38°C	Rash; flushing; urticaria; dyspnea; drug fever - 38°C	Symptomatic bronchospasm, with or without urticaria; allergy related edema/angioedema; hypotension	Anaphylaxis	Death
Acute infusion reaction	Mild reaction; infusion interruption not indicated	Requires therapy or infusion interruption but responds promptly to symptomatic treatment	Prolonged (i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae	Life-threatening; pressor or ventilatory support indicated	Death

Table 2: Grading of cetuximab infusion reaction [40]

1.13 Principle of radiotherapy

Radiation therapy is applied to the cancerous tumor because of its ability to control cell growth. Ionizing radiation works by damaging the DNA of cancerous tissue leading to cellular death. Especially in the head and neck region it is difficult to spare normal tissues, such as mucosa, parotid and submandibular glands, brain and spinal cords. Shaped radiation beams are aimed from several angles of exposure to intersect at the tumour, providing a much larger absorbed dose than in the surrounding, healthy tissue. Besides the tumor itself, the radiation fields may also include the draining of cervical lymph nodes. Uncertainties can be caused by movement of external skin marks relative to the tumor position, therefore patients with carcinomas of the head and neck region need mask adjustment prior to radiation therapy. The majority of epithelial cancers,

including squamous cell carcinoma, are only moderately radiosensitive, and require a significantly higher dose of radiation (60-70 Gray) to achieve a radical cure.[41]

1.13.1 Side effects of radiotherapy

Mucositis usually is the first side effect of radiation. Dental rehabilitation should be done as a prophylaxis before starting with radiation, during treatment regular oral hygiene is recommended and antiseptic rinsing solutions should be used after every meal. Perhaps the most severe side effect of radiation therapy, occurring in three to ten percent of patients, is osteoradionecrosis. Late side effects occur months to years after treatment and are generally limited to the area that has been treated. One frequent long-term effect in the head and neck region is xerostomia, resulting in a higher risk of caries. Long-term side effects are often due to damage of blood vessels and connective tissue cells. Many late effects are reduced by fractionating treatment into smaller parts. [42]

1.14 Prognosis

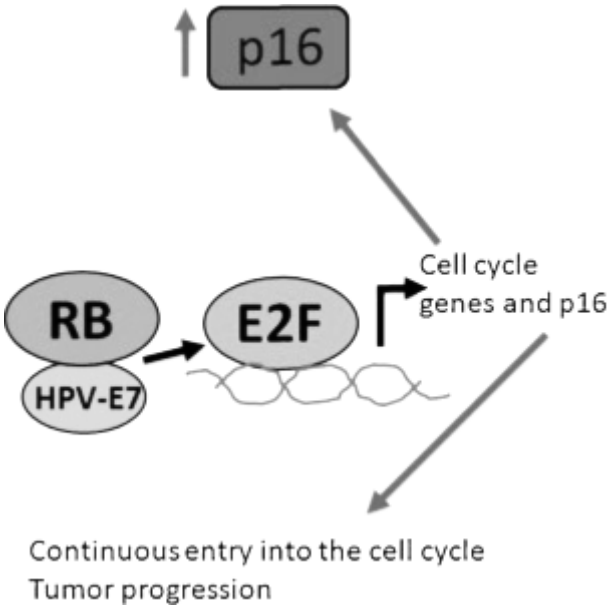
According to Lenarz and Boenninghaus the five-year survival rate for patients with oropharyngeal cancer is T1: 70%, T2: 50%, T3N0: 1-30% and T4: 20%.[43] Patients with HPV positive OPSCC show better prognosis at higher staging. Patients with advanced disease especially with distinct reginal lymph node metastasis, soft tissue metastasis or distant show five-year survival rates <20%. Recent studies at our institute show that patients presenting with small tissue metastasis (STM) at time of first clinical presentation have similarly survival rates as patients presenting with distant metastasis at first visit. Sinha et al have shown that STM was significantly associated with and distant-metastasis free survival (DMFS), but only in the T3-T4, not T1-T2 subset. In patients receiving adjuvant therapy, only high T-classification was associated with DMFS, not STM. Chemoradiation used as adjuvant therapy was not associated with better DMFS in STM patients for any T-classification.[44]

1.15 HPV and p16

As mentioned above, the human papilloma virus (HPV) is an independent risk factor for OPSCC. Kreimer et al. reported in a systematic review of 5046 patients with head and neck squamous cell carcinoma (HNSCC) an overall prevalence of HPV infection of 25.9%, whereas the prevalence of HPV infection was significantly higher among patients with OPSCC (35.6%) than among those with oral (23.5%) or laryngeal (24.0%) squamous cell carcinoma.[45]

In addition to HPV positive status, p16 expression (p16 positive) has been correlated with improved outcome in OPSCC.[46] A functional inactivation of the retinoblastoma protein (pRb) by the HPV E7 protein results in an expression of p16.

HPV viral oncogene products E6 and E7 play a key role in HPV-associated carcinogenesis, blocking p53 and retinoblastoma (Rb) tumor suppressor functions. Rb inactivates E2F, which upregulates cell cycle genes.[47] The functional inactivation of Rb by E7 results in an upregulation of p16, thus HPV positive tumors are characterized by high expression of p16.[48]



Picture 4: Effect of HPV oncogene E7. HPV-E7 abrogates Rb, which usually blocks E2F, resulting in upregulation of cell cycle genes and p16. RB = retinoblastoma protein, HPV = human papilloma virus.[49]

1.16 Inflammation and cancer

The first person to describe a correlation between inflammation and cancer was Rudolf Virchow in 1863. His conclusion, that the tumorigenic process was a result of chronic inflammation, was based on the observation that inflammatory cells often were present in tumor biopsy specimen.[50]

It is known that inflammation is a critical component of tumor progression and many cancers arise from site of infection, chronic irritation and inflammation. According to Alberto Mantovani et al., estimations suggest that 15-20% of all death from cancer worldwide are linked to underlying infections and inflammatory responses.[51,52]

Inflammatory cells orchestrate the tumor microenvironment, which plays a key role in the neoplastic process, fostering proliferation, survival and migration.

Early in the neoplastic process, inflammatory cells are powerful tumor promoters. Aside from producing an attractive environment for tumor growth, these cells facilitate genomic instability and promote angiogenesis. Actions of inflammatory cells, that favor tumor growth, include releasing growth and survival factors, promoting angiogenesis and lymph angiogenesis, stimulation DNA damage, remodeling the extracellular matrix (ECM) to facilitate invasion, coating tumor cells to make available receptors for disseminating cells via lymphatics and capillaries, and evading host defense mechanisms.

Although inflammatory responses should also work against tumor growth, cancer patients are often defective in their inflammatory responses, as a result of two distinct tumor-mediated mechanisms: a desensitization of receptors owing to high chemokine and cytokine concentrations that then blunt systemic responses, resulting in a subversion of the host response, or a failure to upregulate the anti-inflammatory cytokines.

Usually, inflammation is self-limiting, because the production of anti-inflammatory cytokines follows the production of pro-inflammatory cytokines closely. Chronic inflammation on the other hand seems to be due to persistence of the initiating factors or a failure of mechanisms required for resolving the inflammatory response.[53]

In general, two pathways can be described, the intrinsic and the extrinsic pathway.

1.16.1 The intrinsic pathway

Genetic events (e.g. oncogenes, genetic aberrations) causing neoplastic transformation, initiate the expression of inflammation-related programs, triggering the inflammatory cascade, which results in an inflammatory microenvironment.[54]
Ras family oncogenes for example, which are the most frequently mutated dominant oncogenes in human cancer, are able to induce expression and production of inflammatory mediators. As described by Dingzhi Wang et al., *Ras* oncogene transferred into a cervical carcinoma line induces the production of the chemokine CXCL8, which promotes angiogenesis and tumor progression.[55]
Another oncogene, *myc*, instructs remodeling of the extracellular environment with inflammatory cells and mediators playing key roles. In addition to promoting cell autonomous proliferation, the *myc*-activated program also induces several chemokines which recruit mast cells, that have long been known to drive angiogenesis.[56]
Thus, oncogenes representative of different molecular classes, share the capacity to orchestrate pro-inflammatory programs.

1.16.2 The extrinsic pathway

This pathway is driven by inflammatory leukocytes and soluble mediators that induce inflammatory conditions that increase cancer risk. A wide range of chronic infections, exposure to noxious agents that trigger inflammation and auto-immune conditions can be reasons for the implicated inflammatory conditions.[57],[58] Colorectal cancer that develops in patients with inflammatory bowel diseases is the best-established link between chronic inflammation and cancer. Patients with these diseases have a five-to seven-fold increased risk of developing colorectal cancer.[59]
A soluble mediator of cancer-related inflammation is Interleukin-1 (IL-1), which is well established in the involvement of tumor progression. IL-1 expression is elevated in human colon, breast, lung, head and neck cancers and melanomas. IL-1 induces several pro-metastatic genes such as metalloproteinases, chemokines and growth factors and thus promotes tumor growth and metastasis. Moreover, IL-6, which itself is a potent proangiogenic cytokine, is able to stimulate the expression of endothelial adhesion molecules and VEGF (Vascular Endothelial Growth Factor) release is also IL-1 dependent.[60]

A cellular component of cancer-related inflammation is represented by tumor-associated macrophages (TAMs), derived from monocytes that are recruited largely by monocyte chemotactic protein (MCP) chemokines. TAMs may kill neoplastic cells following activation by IL-2, interferon and IL-12[61],[62], but they also produce several potent angiogenic and lymph angiogenic growth factors such as VEGF, cytokines and proteases, all of which are mediators that potentiate neoplastic progression.[63]
IL-10, which effectively blunts the anti-tumor response by cytotoxic T cells, is also produced by TAMs and tumor cells.[64]
Not only do tumor cells take advantage of the trophic factors made by inflammatory cells, but may also use the same adhesion molecules, chemokines and receptors to aid in migration and homing during distant metastatic spread.[65]

Numerous clinical studies of the past years have demonstrated associations between systematic inflammation and poorer prognosis of cancer patients, using inflammation markers like fibrinogen, a nonspecific acute phase protein. [66]

1.16.3 Fibrinogen

In a variety of cancer types the role of pretreatment plasma fibrinogen in predicting survival has been evaluated and confirmed, including renal cell and urothelial cancer[67],[68], endometrial, cervical and ovarian cancer[69],[70],[71], pancreatic cancer[72], colorectal cancer[73],[74], cancer of the gallbladder[75], esophageal[76], lung cancer[77], mesothelioma[78], gastric cancer[79] and hepatocellular carcinoma[80].

The review ‘Prognostic role of pretreatment plasma fibrinogen in patients with solid tumors: A systematic review and meta-analysis’ summarized the data of 52 observational studies and 15,371 patients. The results show a significant association of an elevated baseline plasma fibrinogen with worse overall survival (OS). The highest negative effect of elevated plasma fibrinogen on OS was found in renal cell carcinoma, followed by head and neck cancer and colorectal cancer. Moreover, patients with high baseline fibrinogen had a significantly shorter disease-free survival (DFS) and cancer-specific survival (CSS).[81]

2016 The study ‘Prognostic Impact of Pretreatment Plasma Fibrinogen’ investigated a total of 183 patients with locally advanced oral and oropharyngeal squamous cell cancer (OOSCC) who received neoadjuvant chemoradiation followed by surgery. The study showed that pretreatment plasma fibrinogen predicts response to neoadjuvant chemoradiation: patients with elevated fibrinogen showed significantly higher pathologic stages after neoadjuvant treatment than patients with low fibrinogen (cutoff value of 447 mg/dl). While elevated plasma fibrinogen significantly correlated with higher tumor grade, no association between fibrinogen and clinicopathological parameters, such as age, sex, smoking, clinical T-category and perineural invasion was found.

The study also showed that pretreatment plasma fibrinogen correlates with overall survival and recurrence-free survival: the overall survival at 5 years with elevated fibrinogen was 43,1% and 66,2% for patients in the low fibrinogen group. Additionally, the recurrence-free survival probability at 5 years was 39,4% for patients with elevated fibrinogen and 63,5% for patients in the low fibrinogen group.[82]

2017 The study ‘Plasma Fibrinogen Correlates with Metastasis and is Associated with Prognosis in Human Nasopharyngeal Carcinoma’ of 998 patients showed the following results: Pretreatment plasma fibrinogen is associated with the clinicopathological features of the disease: elevated plasma fibrinogen (cutoff value of 334,5 mg/dl) was associated with older age, advanced TNM stage and patients who developed distant metastasis during follow-up had higher pretreatment plasma fibrinogen than patients who did not develop distant metastasis during follow-up.

Elevated pretreatment plasma fibrinogen is an independent prognostic factor for poor overall survival (OS) and distant-metastasis free survival (DMFS): patients in the high fibrinogen group had poorer 3-years OS and DMFS than patients in the low fibrinogen group (87,5% vs. 94,3%).

Elevated pretreatment plasma fibrinogen is an independent prognostic factor for OS in metastatic patients: the low fibrinogen subgroup achieved better 3-years OS than the high fibrinogen group (85,4% vs. 70,1%). Furthermore, elevated pretreatment plasma fibrinogen was associated with significantly poorer 3-years OS among patients with bone metastasis (48,8% vs. 72,3% for the low fibrinogen group).[83]

1.16.4 CRP (C-reactive protein)

C-reactive protein is an acute phase protein, which is synthesized by the liver and released into the bloodstream after tissue injury within several hours.
Three mechanisms are discussed about the relationship between elevated CRP levels and cancer prognosis:

1. Inflammation causes oxidative damage which promotes tumor growth,
2. CRP release is induced by tumor growth and apoptosis
3. Inflammation is a contributing factor to tumor progression and reflects in elevated CRP levels.[84]

The study 'Roles of preoperative C-reactive protein are more relevant in buccal cancer than in other subsites' of 343 patients with oral cavity squamous cell cancer showed an association between CRP levels and clinicopathological parameters and prognosis: Elevated CRP (cutoff value of 5,0 mg/l) significantly correlated with advanced tumor status, tumor stage, skin invasion, bone invasion, tumor depth and advanced pathologic nodal status and lymphatic invasion.
The study also showed an association between CRP and survival: the group with lower CRP levels had a longer disease-free survival and overall survival than the group with elevated CRP levels.[85]

Although several studies showed significant correlations between elevated pretreatment plasma fibrinogen and poorer prognosis, the results concerning C-reactive protein are quite contradictory. Moreover, the quantity of studies about CRP in oropharyngeal squamous cell carcinoma is low.
Thus, the aim of this work is to evaluate potential prognostic factors in oropharyngeal squamous cell carcinoma, with focus on pretreatment plasma fibrinogen and pretreatment C-reactive protein, but also their development during treatment.

2. Methods

The current study is a single-center, retrospective observational study with a consecutive cohort including patients who were treated at the Clinical Division of ENT, Medical University of Graz, Austria. These patients were drawn from our in-house head and neck cancer cohort, which includes around 720 patients who were seen at our department since January 2011 until December 2015. The mean age at first diagnosis was 64.1 ± 12.86 years and 26.6% of the patients were female. More than half (52.3%) of the cohort presented with an ECOG stage of 0, 31.8% presented with ECOG 1 and the remaining 15.9% presented with an ECOG stage higher than 1. The most frequent tumor entity was the Squamous Cell Carcinoma (82.5%), followed by Adenocarcinoma (3.4%). The most frequent tumor sites were the oropharynx, the larynx and the oral cavity.

Patients suitable for inclusion in this study had to meet the following criteria: location of the primary tumor in the oropharynx, histological confirmed squamous cell carcinoma and age of patients older than 18 years. Of the remaining 161 patients, 39 were female (24,2%) and 122 males (75,8%). The mean age at first diagnosis was 61 ± 10.25 years.
The mean age at death was 62 ± 10.58 years. The mean follow-up was 31 ± 23.2 months.

The cohort was divided into four groups, dependent on the localization of the tumor:

- Tonsils: Group A
- Oropharynx: Group B
- Root of the tongue: Group C
- Soft palate: Group D

Also, the cohort was divided into two groups, whether surgery was performed or not.
Whether the tumor was p16 associated or not was tested not before the midyear of 2013, so only 25 of 161 patients could be separated into a p16-positive or p16-negative group.
Thus, the old AJCC (2009) classification was used for this cohort. Of 14 patients, the AJCC data was missing, of the remaining 185, 4 patients were classified with a G1

carcinoma (2,2%), 70 patients with G2 (37,8%), 105 patients with G3 (56,8%), 3 patients with G4 (1,6%) and in 3 patients the classification was not possible (1,6%).

2.1 Statistical methods and analysis

Primary endpoints were overall survival (OS) and disease-free survival (DFS) during a mean follow-up of 31 ± 23.2 months. Assessed variables were Patient ID, date of birth, postal, sex, date of death, date of first diagnosis, date of first tumor board council meeting, tumor localization, tumor side, TNM stage, histological entity, keratotic, p16, comorbidities (pulmonological, cardiac, renal, liver, psychological, neurological, hematological, others), primary path of suggested therapy (curative, palliative, best supportive care), primary therapy (surgery, radiation, chemotherapy), progression, recurrence, secondary tumor, metastasis, labor1 (time at first diagnosis (leukocytes, erythrocytes, thrombocytes, hemoglobin, neutrophils, monocytes, lymphocytes, GGT, AST, ALT, LDH, CRP, fibrinogen) and labor2 (three months after diagnosis).

Data analysis was performed using SPSS software (version 24.0; IBM SPSS Statistics). Data are described as medians with ranges or means with standard deviations, as appropriate. Comparisons of continuous or categorical variables were performed with Student's t-test or Mann–Whitney-U test, and Chi-squared tests or Fisher exact test, as appropriate. Statistical significance was set at P=0.05. Comparisons were made between subgroups. Kaplan-Meier analysis was used to estimate recurrence free survival and overall survival. Significances are identified by log-rank test. Cox - proportional hazard modelling was used to analyze risk-predicting recurrences or death.

The study was approved by the Ethics Committee of the Medical University of Graz.

3. Results

3.1 Demographics

Hundred-sixty-one patients were included in this analysis, 39 were female (24,2%) (Figure 5). The median age of the cohort at first diagnosis was 61 ± 10.25 years. The mean follow-up was 31 ± 23.2 months. The mean age at death was 62 ± 10.58 years. During follow-up 45 (28%) patients died, 37 (82%) was a cancer related death. Reasons for non-cancer related deaths were cardiac insufficiency and stroke.

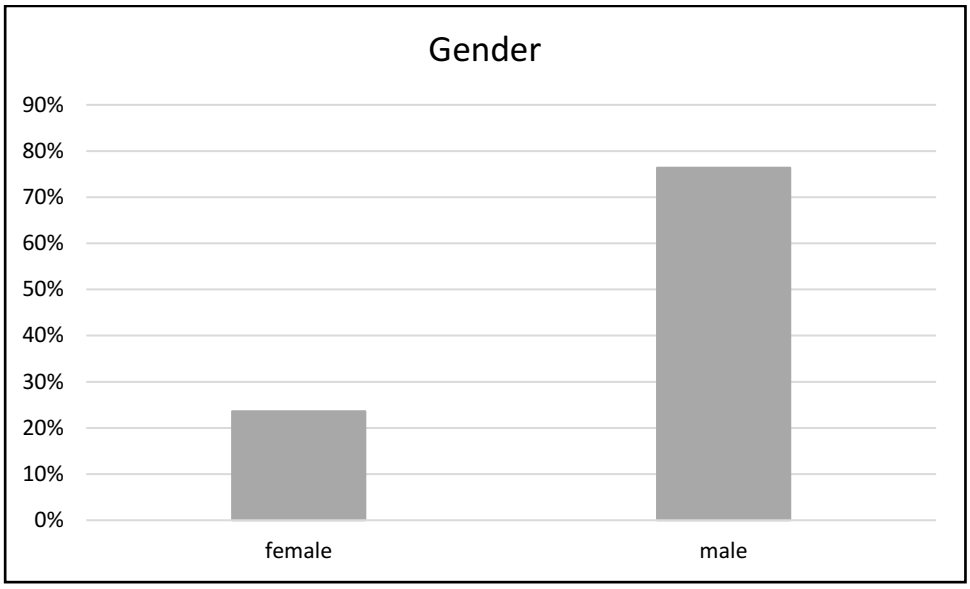


Figure 5: Distribution of gender in OPSCC

3.2 Tumor localizations

The most frequent tumor sites were tonsils (n=92 (57,1%)), followed by root of the tongue (n=36 (22,4%)) and oropharyngeal wall (n=33 (20,5%)) (Figure 6).

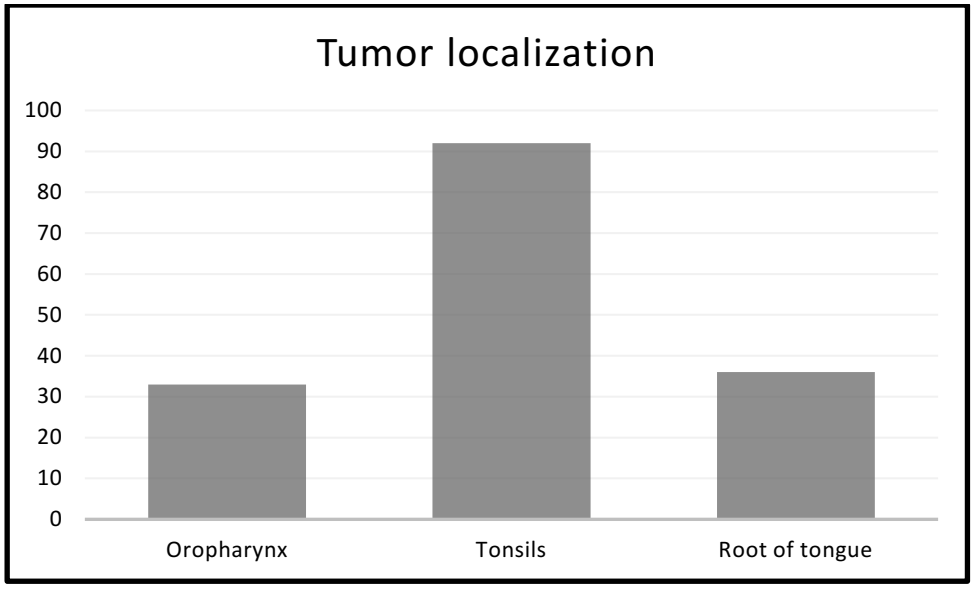


Figure 6: Tumor localization of OPSCC

3.3 TNM-classification - AJCC 7th edition

In this study the AJCC 7th edition classification was used for primary staging. T-classification: Of 161 patients, 23 (14,3%) presented with a T1 tumor, 31 (19,3%) with a T2, 36 (22,4%) with a T3, and 71 (44,1%) presented with a T4 tumor (Figure 7).

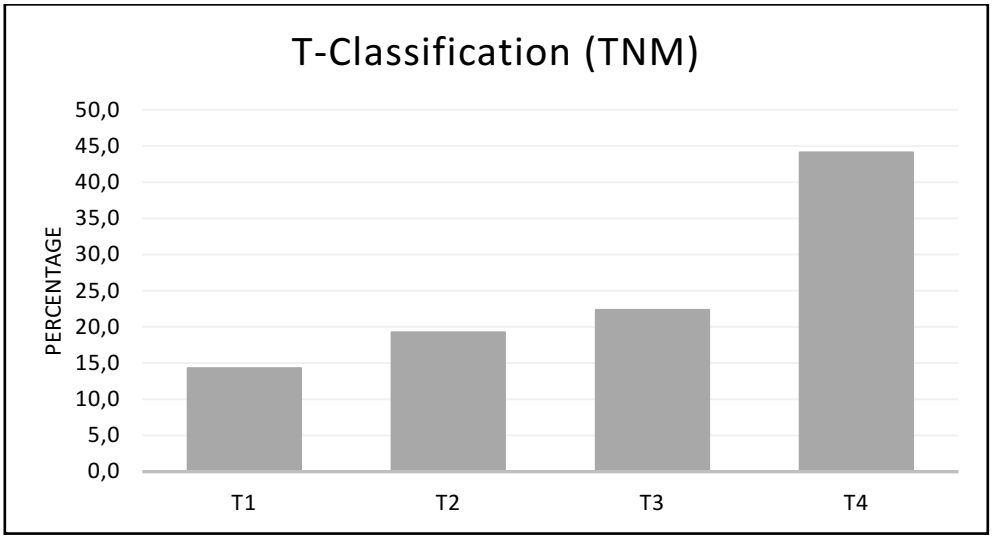


Figure 7: T-classification (AJCC 7th edition)

N-classification: 62 (39%) patients showed lymph node metastasis degree of N2b as largest group. Only 23% of patients with OPSCC presented without cervical lymph node metastasis (N0) (Figure 8).

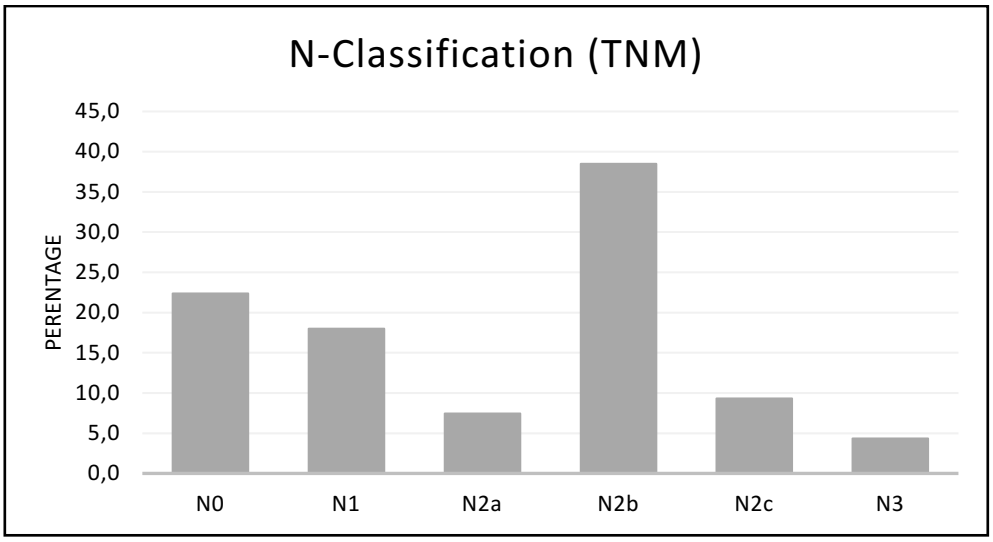


Figure 8: N-classification (AJCC 7th edition)

M-classification: 150 (93%) patients did not show distant metastases at first clinical presentation (Figure 9).

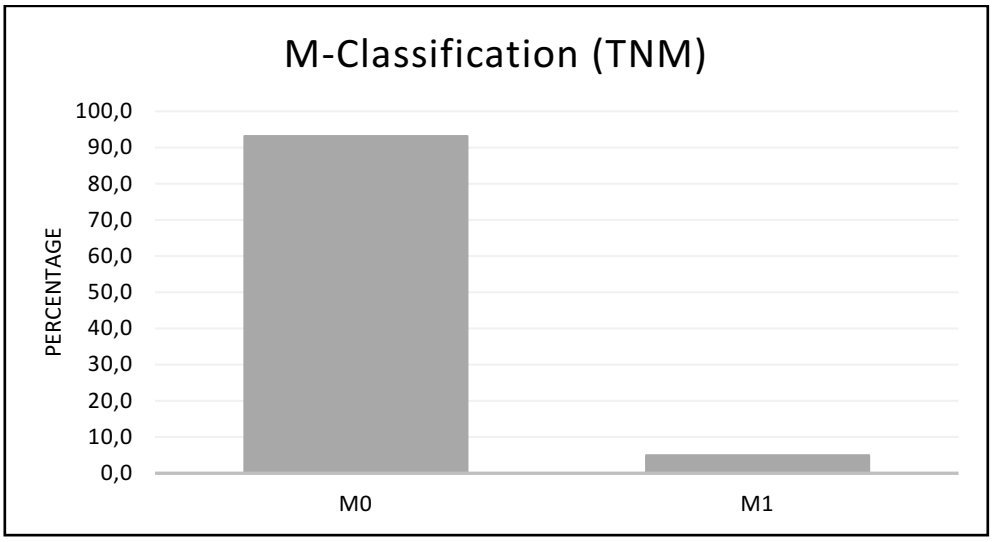


Figure 9: M-classification (AJCC 7th edition)

Of 161 patients, the majority presented with low differntiated (G3) scquamous cell carcinoma (n=96 (62,7%)). The date of 8 patients was missing, and for 3 patients grading could not be assessed (Figure 10).

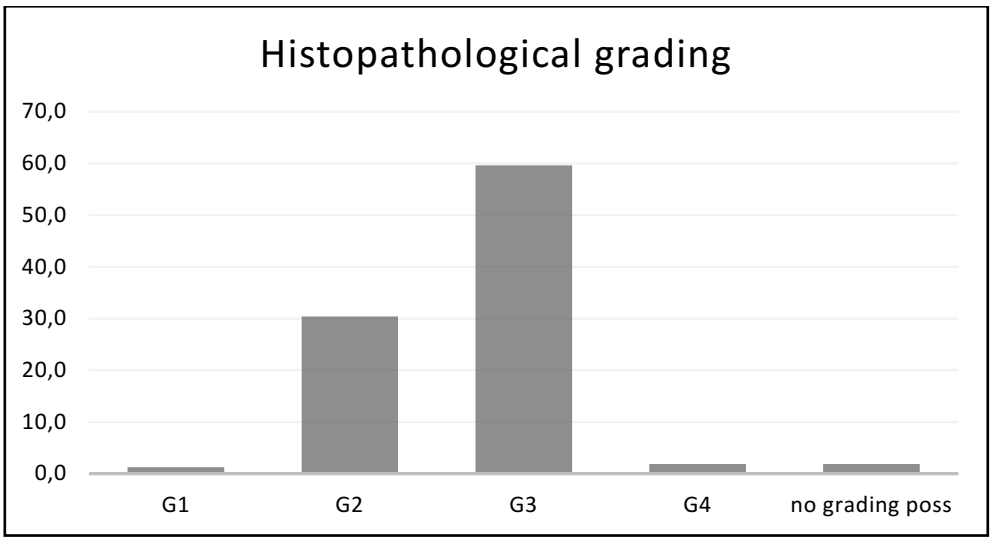


Figure 10 : pathological grading of SCC

Abbreviations: G1 = Well differentiated (Low grade); G2 = Moderately differentiated (Intermediate grade); G3 = Poorly differentiated (High grade), G4= Undifferentiated (High grade);

Most patients (64%) presented already with N2b cervical lymph node metastasis, thus were staged as IVA at a minimum (Figure 11).

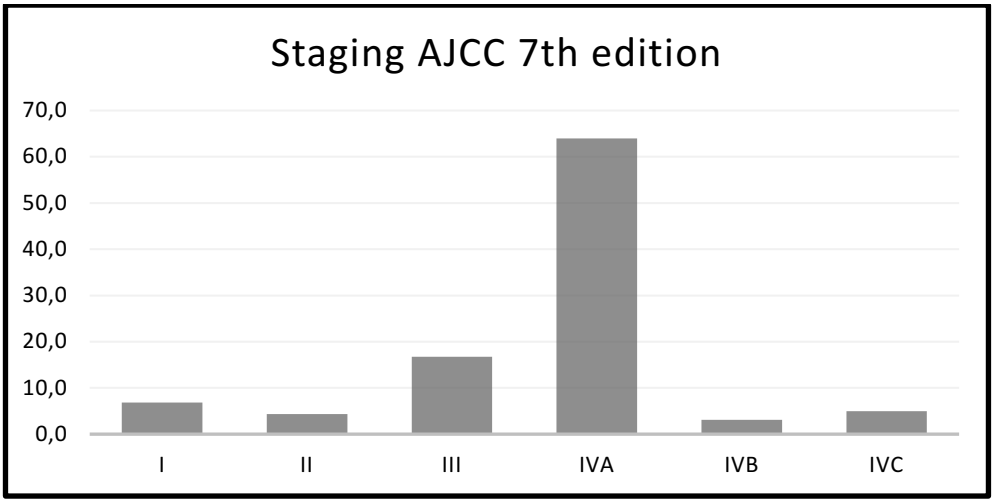


Figure 11: Staging – AJCC 7th edition

Abbreviations: I = localized, II = early locally advanced, III = late locally advanced, IVA-C=inoperable or distant metastasized

3.4 ECOG Scale

Of all 161 patients the ECOG Performance Status was evaluated. 96 patients (59,6%) presented with an ECOG of 0, 47 patients (29,2%) with ECOG 1, and 18 patients (11,2%) with ECOG >1 (Figure *12*).

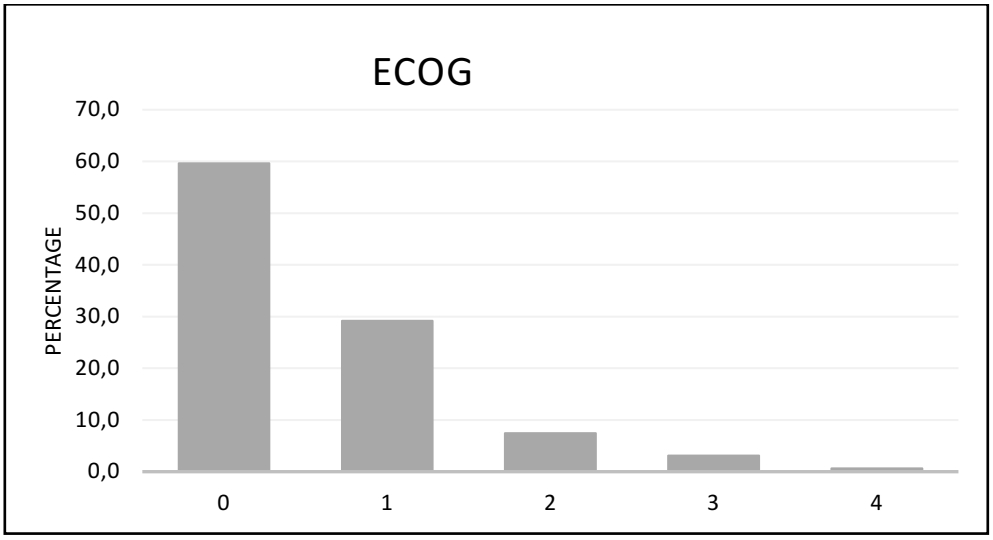


Figure 12: ECOG Performance score

3.5 Primary path of suggested therapy

Of 161 patients, the primary path of therapy of 126 (78,3%) patients was curative, of 20 (12,4%) patients palliative, and of 14 (8,7%) patients the primary path of therapy was best supportive care. The data was missing of 1 (0,6%) patient (Figure 13).

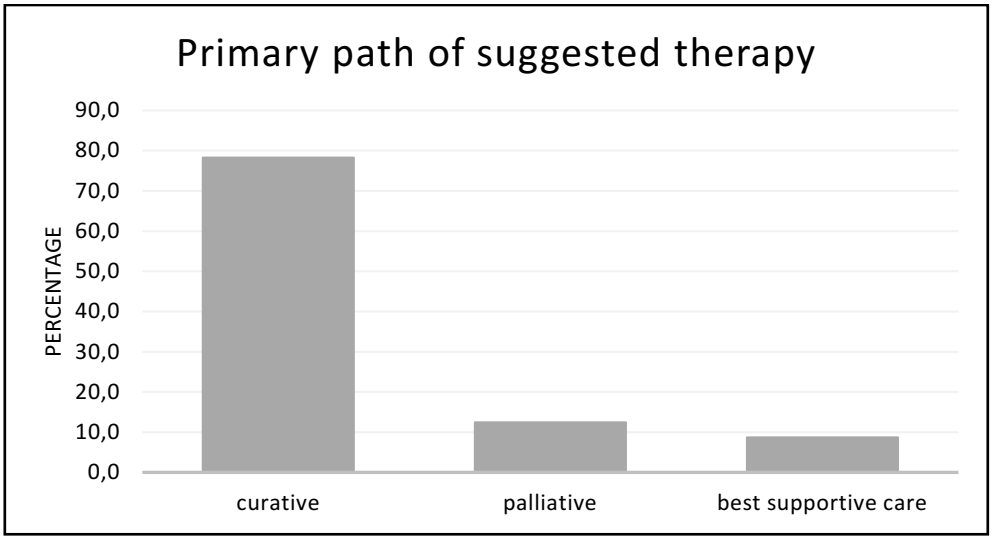


Figure 13: Primary path of therapy

Detailed information concerning the study population and the tumor are summarized in (Table 3).

Variable	N (%miss.)	Frequency	%
gender	161	100%	
female		39	24%
male		122	76%
T-class.	161	100%	
T1		23	14%
T2		31	19%
T3		36	22%
T4a		71	44%
M-class.	158 (2%)	98%	
M0		150	95%
M1		8	5%
Histopatho. grading	153 (5%)	95%	
G1		2	1%
G2		49	32%
G3		96	63%
G4		3	2%
GX		3	2%
Progression	161	100%	
yes		67	42%
no		94	58%
Distant metastasis	161	100%	
yes		16	10%
no		145	90%

Variable	N (%miss.)	Frequency	%
ECOG	161	100%	
0		96	60%
1		47	29%
2		12	7%
3		5	3%
4		1	1%
N-class.	161	100%	
N0		36	22%
N1		29	18%
N2a		12	7%
N2b		62	39%
N2c		15	9%
N3		7	4%
Tumor stage	161	100%	
I		11	7%
II		7	4%
III		27	17%
IVa		103	64%
IVb		5	3%
IVc		8	5%
Local recurrence	160 (1%)	99%	
yes		14	9%
no		146	91%
Second malignoma	156 (3%)	97%	
yes		14	9%
no		142	91%

Table 3: Characteristics of the study population.

The column “N (%miss.)” indicates the number of patients with observed values of the respective variable (% missing), the categorical variables in the column “summary measure” are reported as absolute frequencies and percentages.

3.6 Follow up and cancer related death

The mean follow-up was 31 ± 23.2 months during which 37 patients (23%) were lost in follow-up. At the end of follow-up 79 (49,1%) patients were alive, 45 (28%) patients died (Figure 14), of those 45 patients, 37 (82%) deaths were cancer related (Figure 15).

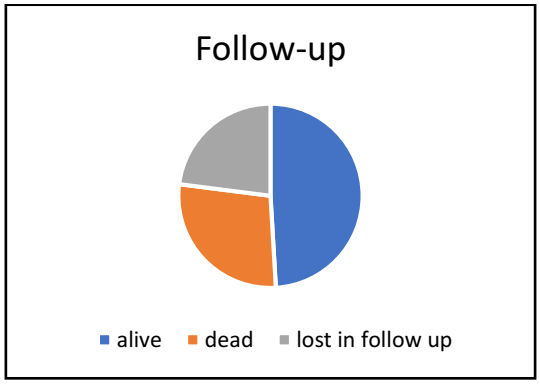


Figure 14: Follow-up

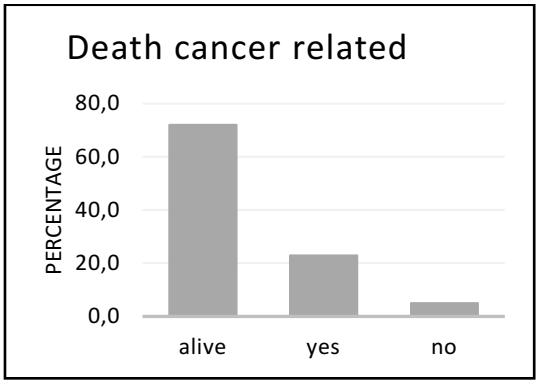


Figure 15: Death related cancer

3.7 Association between T – classification and OS/DFS

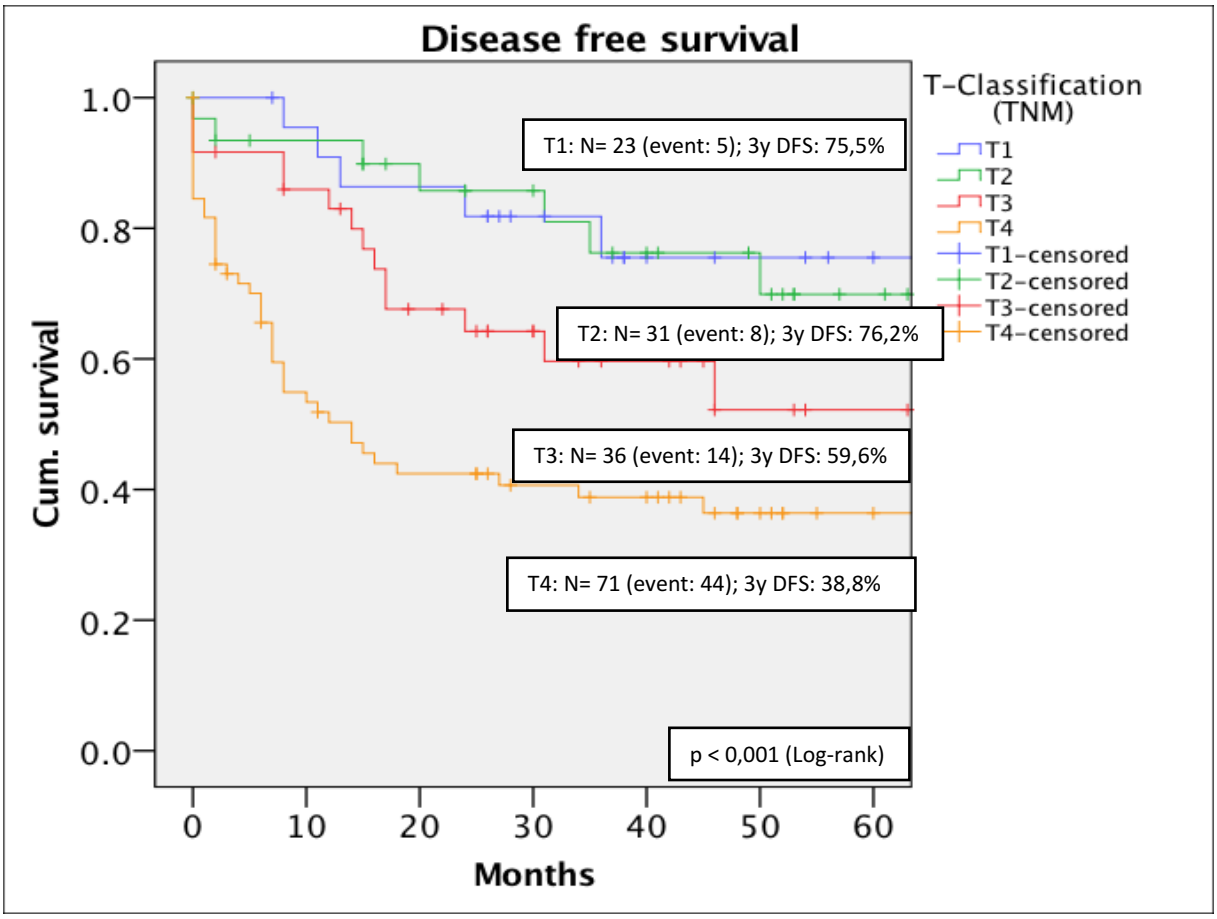


Figure 16: T-classification and DFS in OPSCC

Abbreviation: 3y DFS = 3-year disease-free survival

The mean DFS for T1-carcinoma, represented by the blue line, was 65,9±5,9 months (CI: 54,3-77,5).

The mean DFS for T2-carcinoma, represented by the green line, was 58,1± 4,6months (CI: 48,9-67,1).

The mean DFS for T3-carcinoma, represented by the red line, was 50,6±5,9months (CI: 38,9-62,3).

The mean DFS for T4-carcinoma, represented by the yellow line, was 31,2±3,9months (CI: 23,5-38,8).

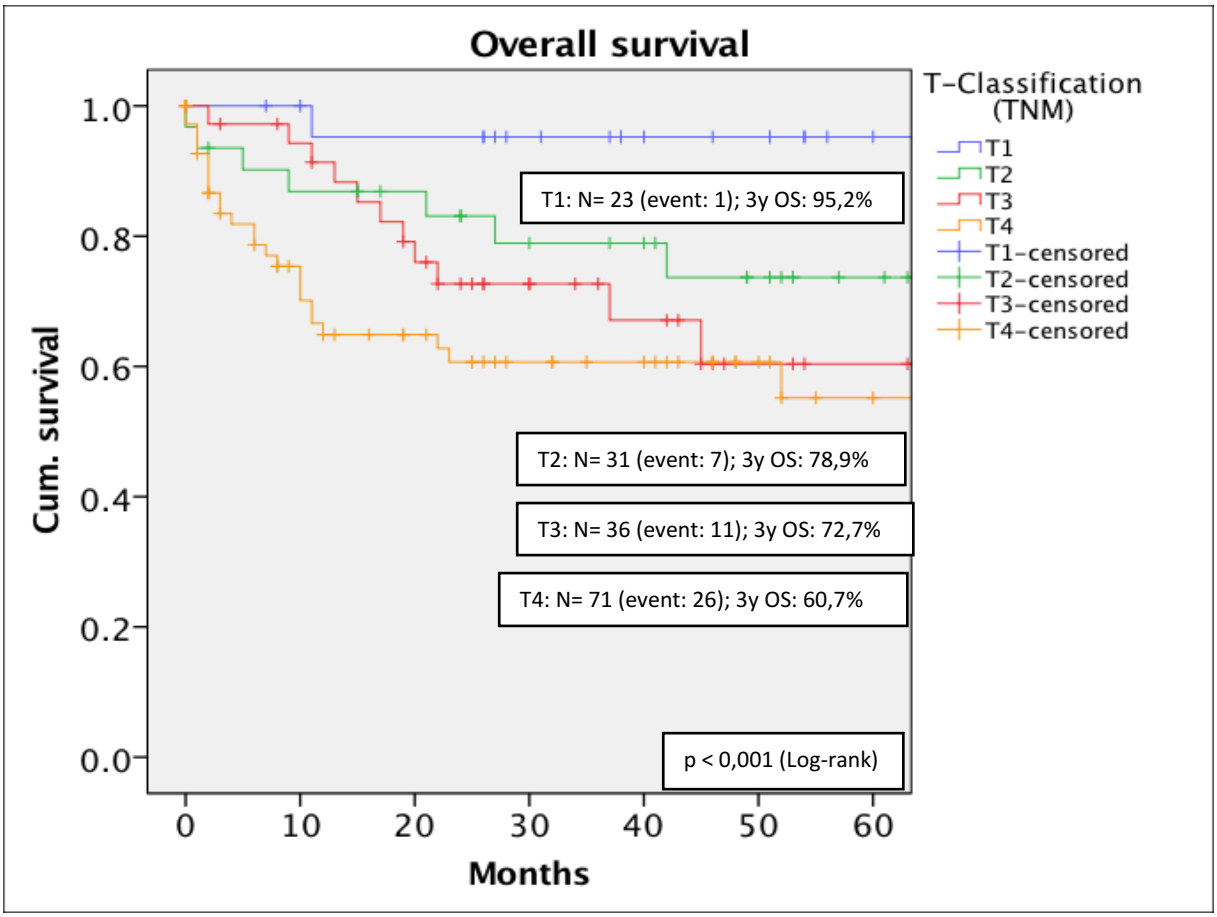


Figure 17: T-classification and OS in patients with oropharyngeal cancer

Abreviations: 3y OS = 3-year overall survival

The mean OS for T1-carcinoma, represented by the blue line, was 77,7± 3,2months (CI: 71,3- 84,0).

The mean OS for T2-carcinoma, represented by the green line, was 58,4± 4,8months (CI: 48,8-67,9).

The mean OS for T3-carcinoma, represented by the red line, was 57,3±5,5months (CI: 46,4- 68,2).

The mean OS for T4-carcinoma, represented by the yellow line, was 46,9± 4,3months (CI: 38,3- 55,4).

3.8 Association between tumor localization and OS/DFS

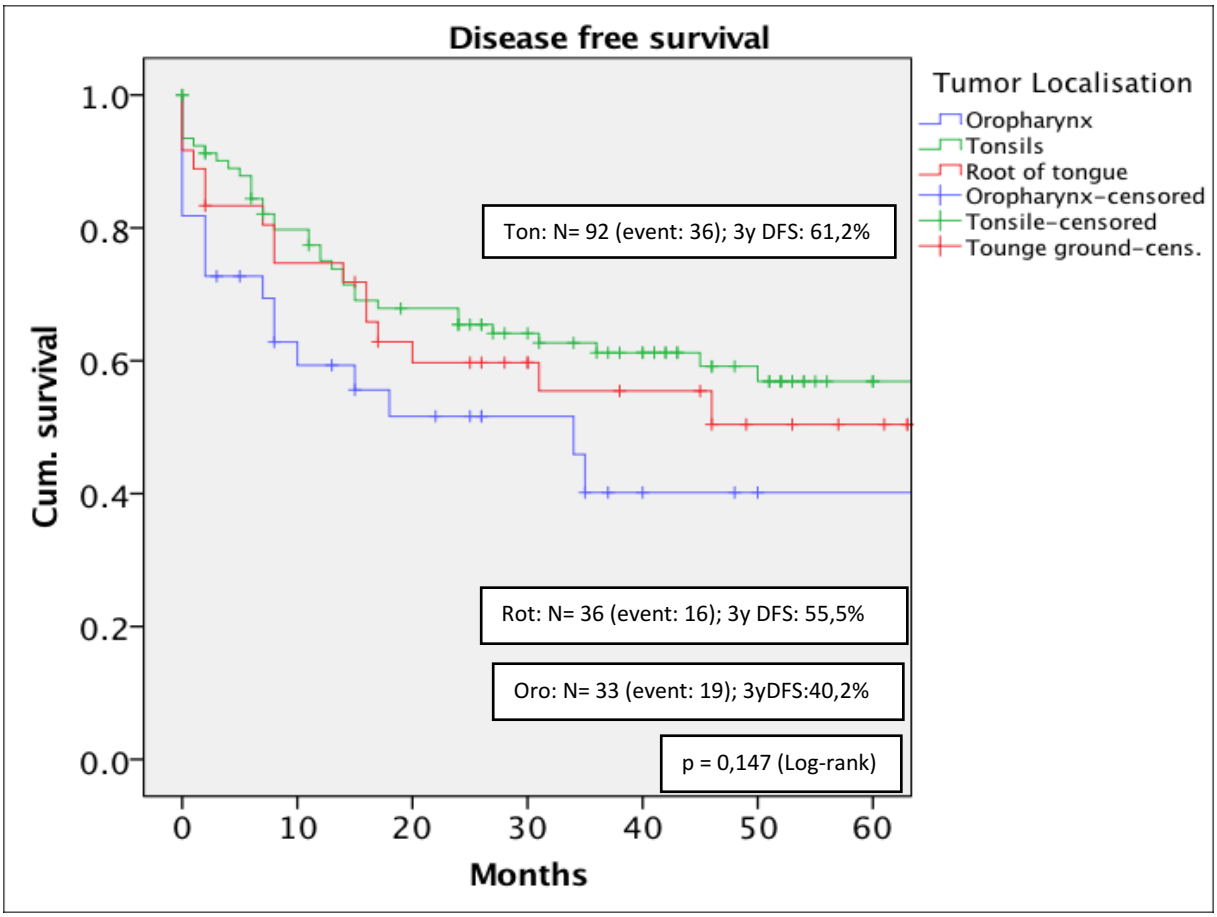


Figure 18: Tumor localization and DFS in patients with oropharyngeal cancer;

Abreviations: Ton = Tonsil group, Rot = Root of the tongue group, Oropharynx = Oropharynx group, 3y DFS = 3-year disease-free survival

The mean DFS in the tonsil group, represented by the green line, was 51,6± 3,7months (CI: 44,2- 58,9).

The mean DFS in the root of the tongue group, represented by the red line, was 44,2±5,5 months (CI: 33,4- 55,0).

The mean DFS in the oropharynx group, represented by the blue line, was 32,8±5,5 months (CI: 22,0- 43,6).

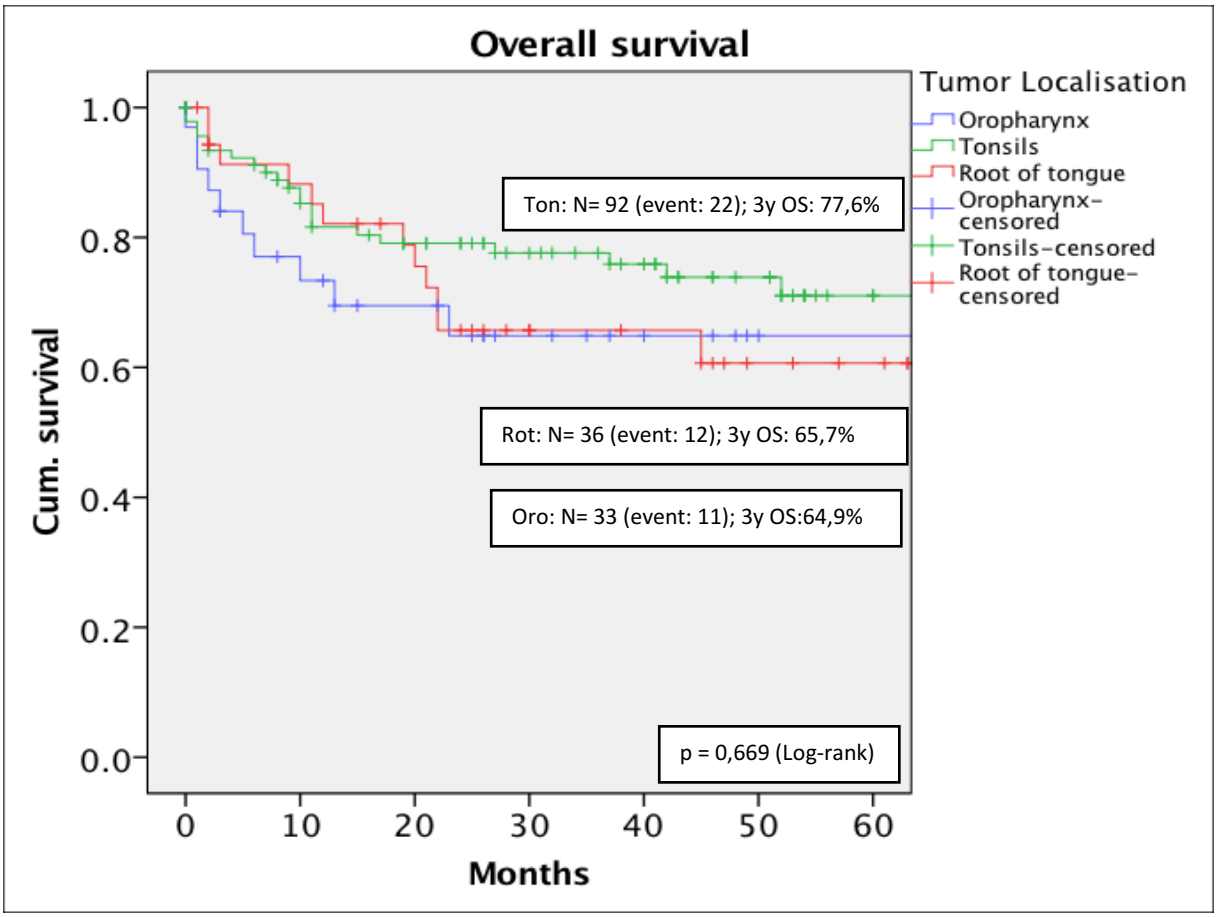


Figure 19: Tumor localization and OS in patients with oropharyngeal cancer;

Abreviations: Ton = Tonsil group, Rot = Root of the tongue group, Oropharynx = Oropharynx group, 3y OS = 3-year overall survival

The mean OS in the oropharynx group, represented by the blue line, was 48,9± 6,2months (CI: 36,8- 61,1).

The mean OS in the root of the tongue group, represented by the red line, was 51,7 ± 5,1 months (CI: 41,6 - 61,8).

The mean OS in the tonsils group, represented by the green line, was 62,4 ± 3,4 months (CI: 55,6 - 69,1).

3.9 Analysis of CRP and the association with OS and DFS

The mean baseline CRP was 17,7 mg/dl; the mean baseline CRP in the female group was 19,6 mg/dl and 17,1 mg/dl in the male group.

Using ROC curve analysis, a pretreatment CRP cutoff value of 13,6 mg/dl was determined (Figure 11). At this cutoff point sensitivity was 0,438, specificity was 0,861 and area under the curve (AUC) was 0,673. CRP levels equal to or below the cutoff point were considered low (n=102/143 patients), while CRP levels above the cutoff point were considered high (n=41/143 patients).

3.9.1 Overall survival - CRP

3.9.1.1 Total cohort

The total cohort showed highly significant differences in overall survival (OS) in the low CRP vs. the high CRP group (p =0,003) (Figure 20).
In the CRP group <13.6 mg/dl the mean OS was 63,1±3,2 months (confidence interval [CI]: 56,8-69,3). The CRP group >13.6 mg/dl showed a mean OS of 42,1±5,7 months (CI: 30,8-53,3).

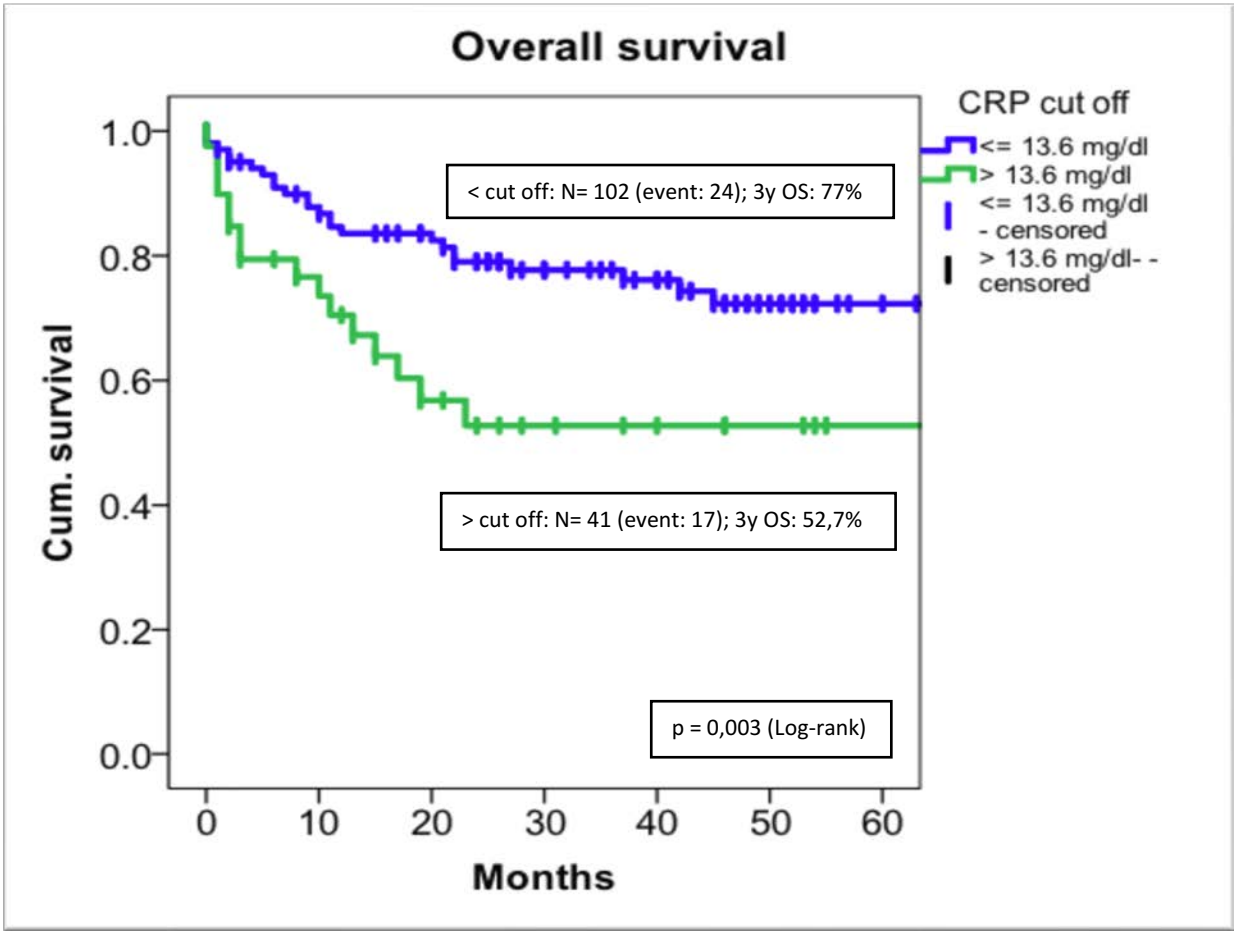


Figure 20: Overall survival in patients with oropharyngeal cancer - total cohort;

Abbreviations: 3y OS = 3-year overall survival

3.9.1.2 Oropharynx

The oropharynx group showed significant differences in OS in the low CRP vs. the high CRP group (p =0,023) (Figure 21).
In the CRP group <13.6 mg/dl the mean OS was 41,7±4,2 months (CI: 33,3-50,2). The CRP group >13.6 mg/dl showed a mean OS of 32,5±9,7 months (CI: 13,4-51,6).

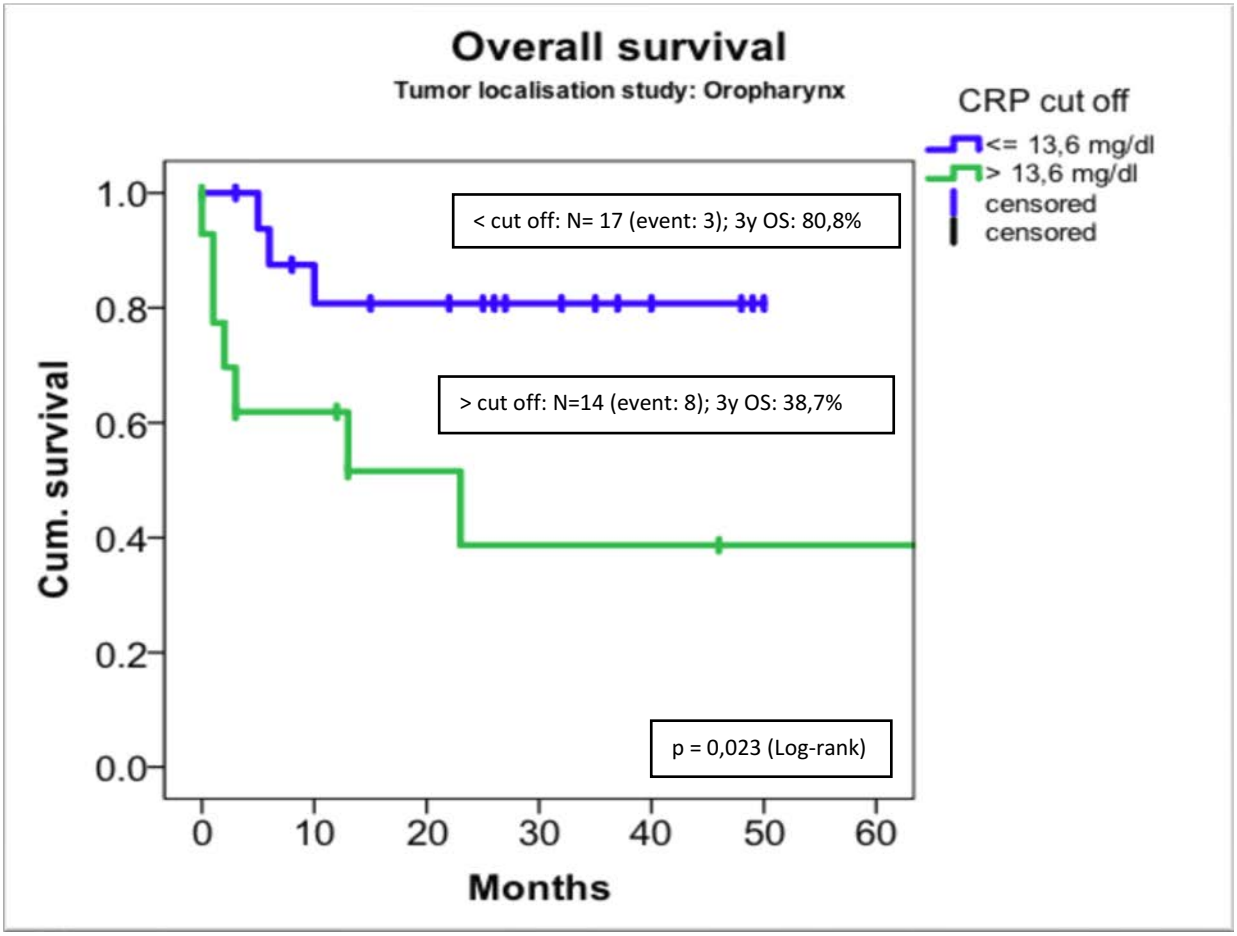


Figure 21: Overall survival in patients with oropharyngeal cancer: Oropharynx

Abbreviations: 3y OS = 3-year overall survival

3.9.1.3 Tonsils

The tonsils group did not show significant differences in OS in the low CRP vs. the high CRP group (p =0,398) (Figure 22).

In the CRP group <13.6 mg/dl the mean OS was 64,3±4,1 months (CI: 56,3-72,3). The CRP group >13.6 mg/dl showed a mean OS of 39,8±5,1 months (CI: 29,8-49,8).

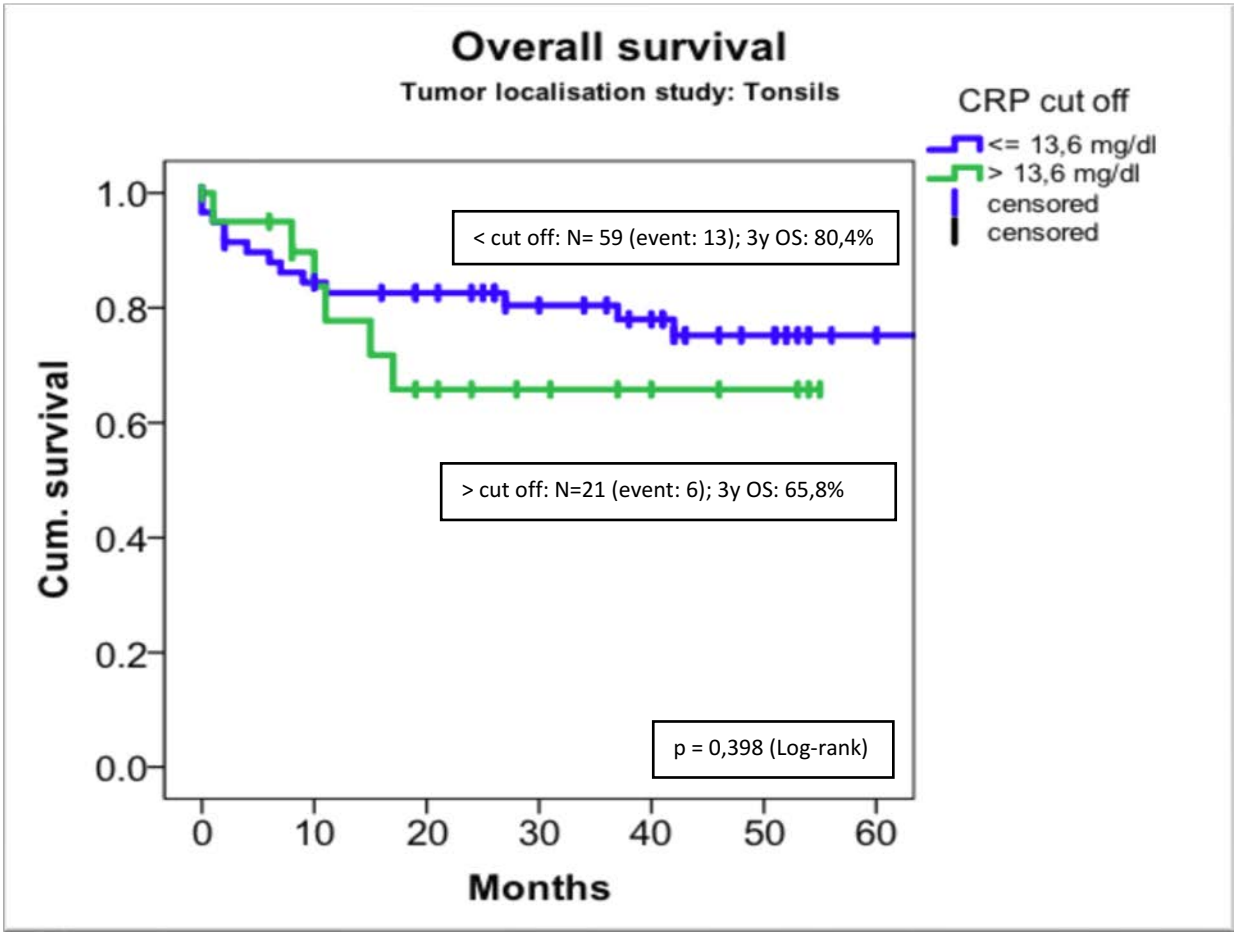


Figure 22: Overall survival in patients with oropharyngeal cancer: Tonsils;

Abbreviation: 3y OS = 3-year overall survival

3.9.1.4 Root of the tongue

The root of the tongue group showed significant differences in OS in the low CRP vs. the high CRP group (p =0,029) (Figure 23).
In the CRP group <13.6 mg/dl the mean OS was 55,0±5,5 months (CI: 44,2-65,7). The CRP group >13.6 mg/dl showed a mean OS of 15,0±4,5 months (CI: 6,2-23,9).

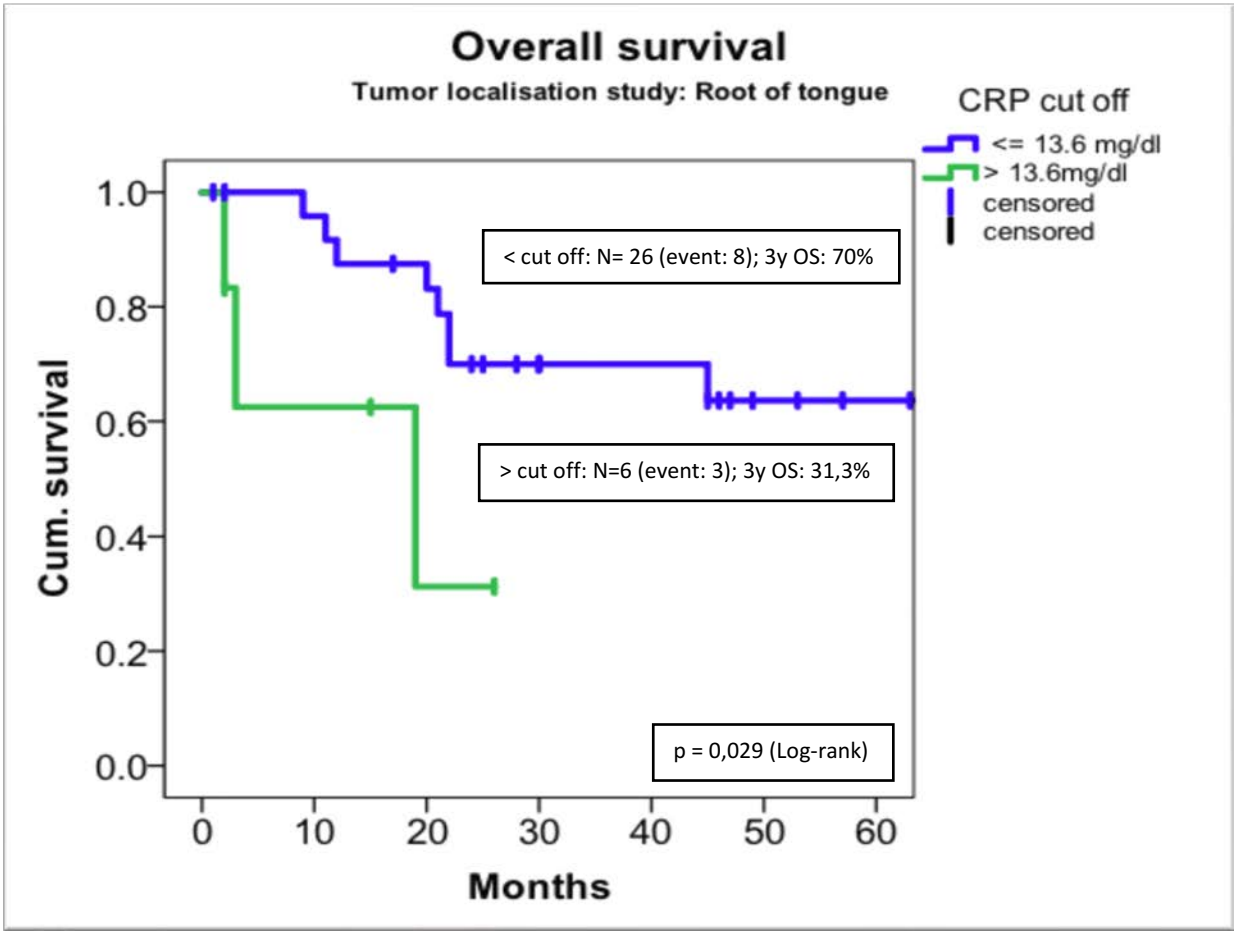


Figure 23: Overall survival in patients with oropharyngeal cancer: Root of the tongue

Abbreviations: 3y OS = 3-year overall survival

T3 & T4 group

The group with a T3 - carcinoma showed significant differences in OS in the low CRP vs. the high CRP group (p =0,04) (Figure 24).
In the CRP group <13.6 mg/dl the mean OS was 60,5±6,2 months (CI: 48,4-72,6). The CRP group >13.6 mg/dl showed a mean OS of 26,0±5,8 months (CI: 14,6-37,4).

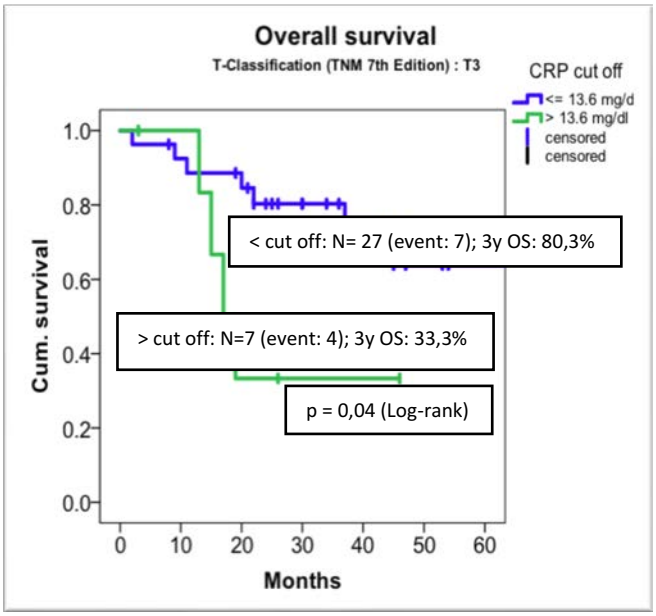


Figure 24: Overall survival in patients with oropharyngeal cancer: T3-carcinoma

Abbreviation: 3y OS = 3-year overall survival

Also, the group with a T4 - carcinoma showed significant differences in OS in the low CRP vs. the high CRP group (p =0,05) (Figure 25).
In the CRP group <13.6 mg/dl the mean OS was 53,9±5,3 months (CI: 43,5-64,4). The CRP group >13.6 mg/dl showed a mean OS of 36,8±7,7 months (CI: 21,8-51,9).

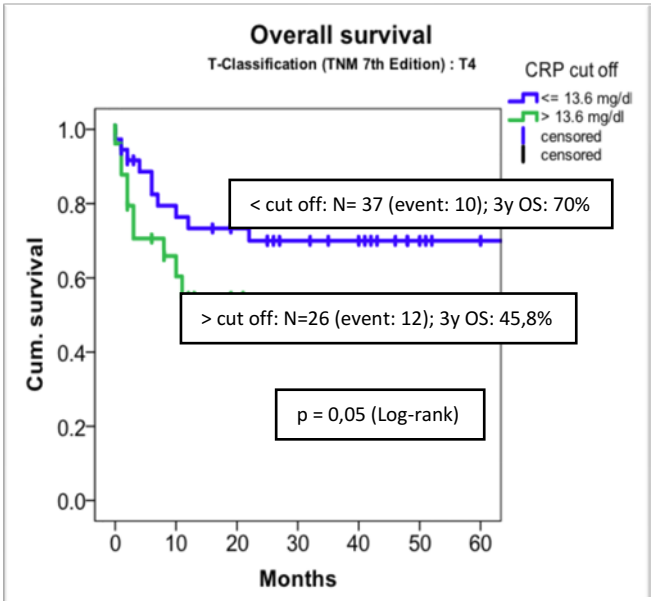


Figure 25: Overall survival in patients with oropharyngeal cancer: T4-carcinoma

Abbreviations: 3y OS = 3-year overall survival

:

3.9.2 Disease free survival - CRP

Total cohort

The total cohort showed highly significant differences in disease free survival (DFS) in the low CRP vs. the high CRP group (p <0,001) (Figure 26).
In the CRP group <13.6 mg/dl the mean DFS was 55,1±3,5 months (CI: 48,4-68.9).
The CRP group >13.6 mg/dl showed a mean DFS of 25,2±4,8 months (CI: 16,2-34,1).

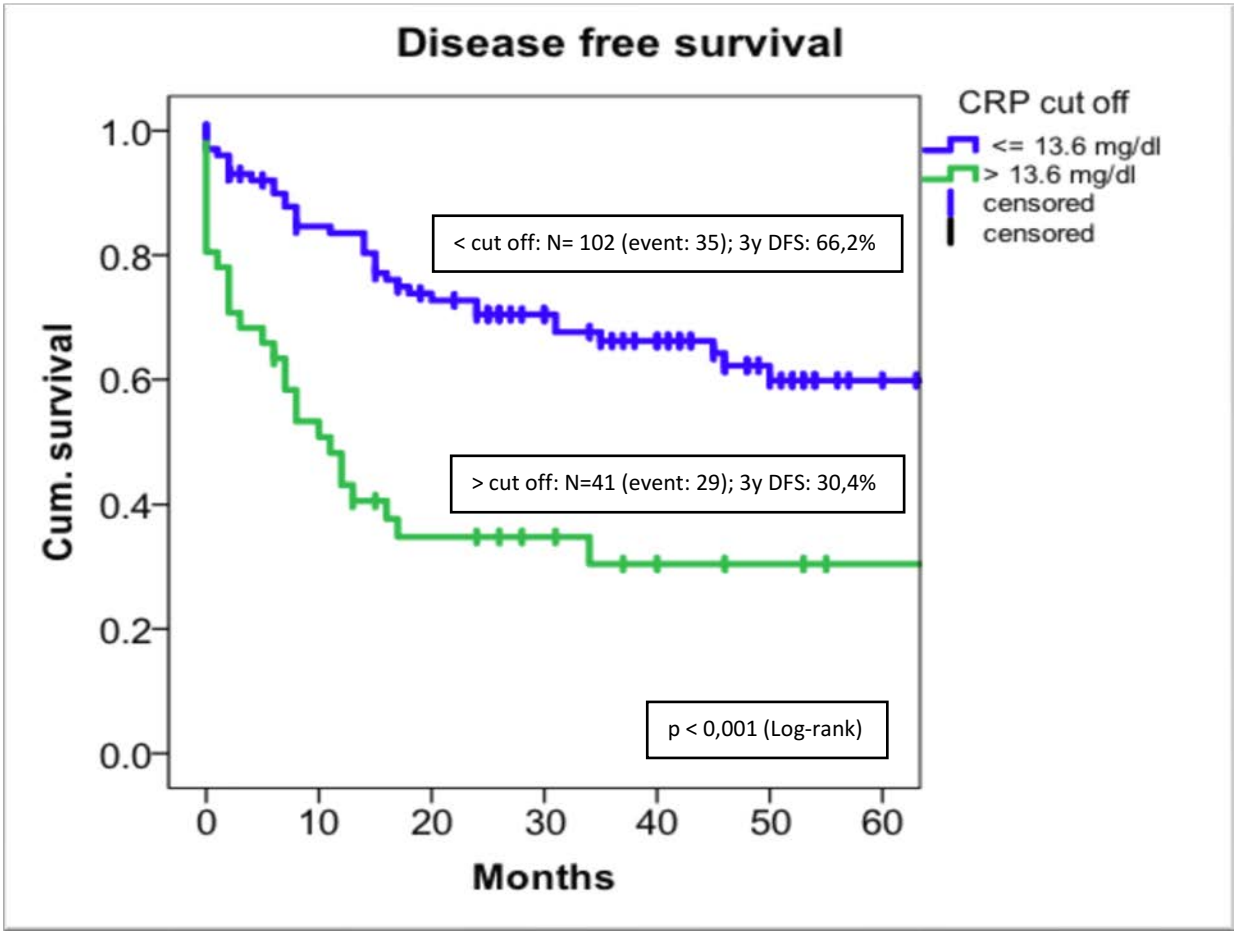


Figure 26: Disease free survival in patients with oropharyngeal cancer: Total cohort

Abbreviations: 3y DFS = 3-year disease-free survival

3.9.2.1 Oropharynx – disease free survival

The oropharynx group showed significant differences in DFS in the low CRP vs. the high CRP group (p =0,003) (Figure 27).
In the CRP group <13.6 mg/dl the mean DFS was 37,4±4,6 months (CI: 28,2-46,5). The CRP group >13.6 mg/dl showed a mean DFS of 17,9±7,1 months (CI: 3,9-31,2).

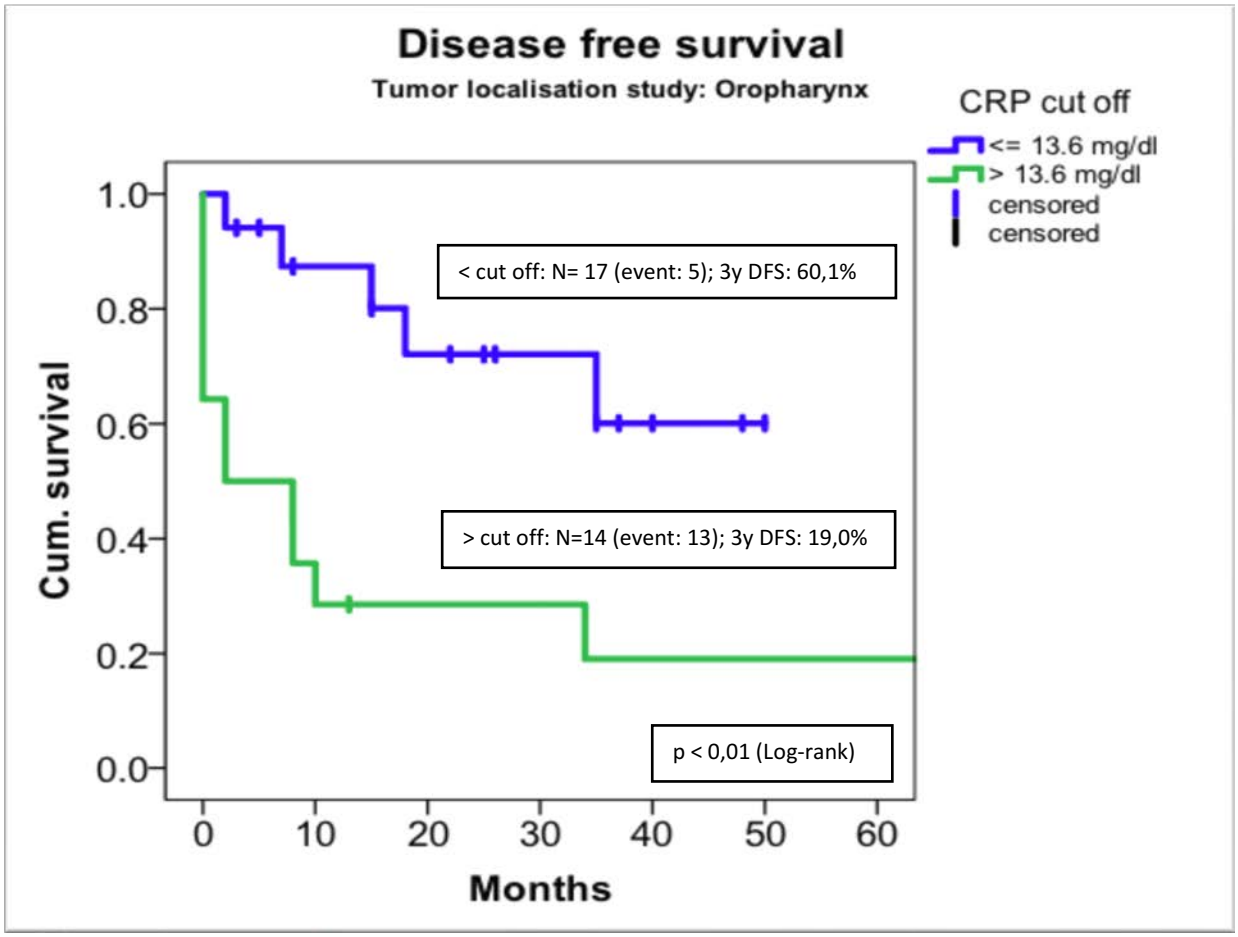


Figure 27: Disease free survival in patients with oropharyngeal cancer: Oropharynx

Abbreviations: 3y DFS = 3-year disease-free survival

3.9.2.2 Tonsils – disease free survival

The tonsil group showed significant differences in DFS in the low CRP vs. the high CRP group (p =0,011) (Figure 28).

In the CRP group <13.6 mg/dl the mean DFS was 57,4±4,4 months (CI: 48,8-67,2). The CRP group >13.6 mg/dl showed a mean DFS of 27,0±5,2 months (CI: 16,8-37,3).

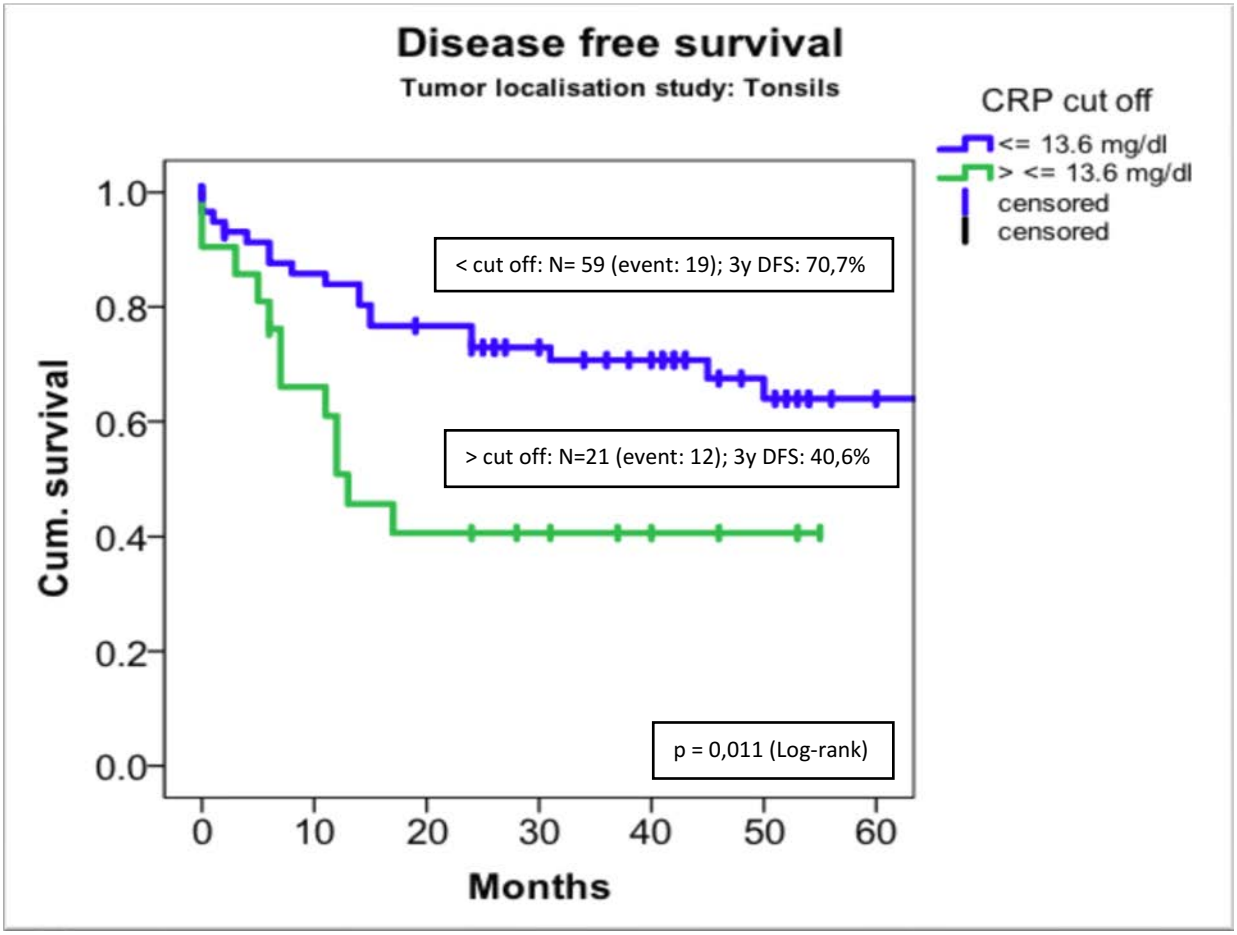


Figure 28: Disease free survival in patients with oropharyngeal cancer: Tonsil

Abbreviations: 3y DFS = 3-year disease-free survival

3.9.2.3 Root of the tongue — disease free survival

The root of the tongue group showed significant differences in DFS in the low CRP vs. the high CRP group (p =0,039) (
Figure *29*).
In the CRP group <13.6 mg/dl the mean DFS was 46,8±6,2 months (CI: 34,5-58,9). The CRP group >13.6 mg/dl showed a mean DFS of 11,0±4,5 months (CI: 2,3-19,7).

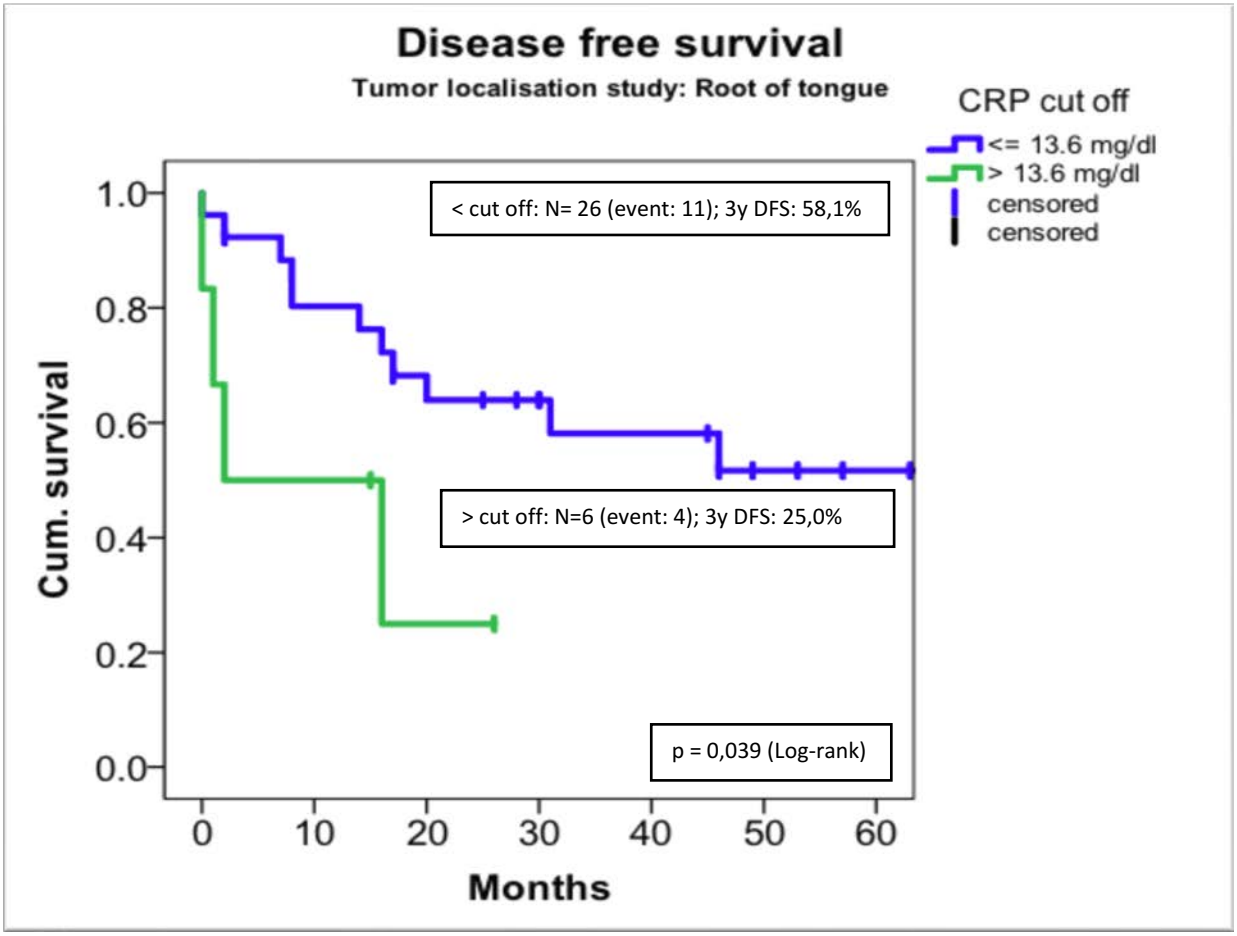


Figure 29: Disease free survival in patients with oropharyngeal cancer: Root of the tongue

Abbreviation: 3y DFS = 3-year disease-free survival

3.9.2.4 T4 – group – disease free survival

The group of patients with a T4- carcinoma showed highly significant differences in DFS in the low CRP vs. the high CRP group (p <0,001) (
Figure *30*).
In the CRP group <13.6 mg/dl the mean DFS was 43,7±5,6 months (CI: 32,6-54,6).
The CRP group >13.6 mg/dl showed a mean DFS of 15,4±4,7 months (CI: 6,2-27,7).

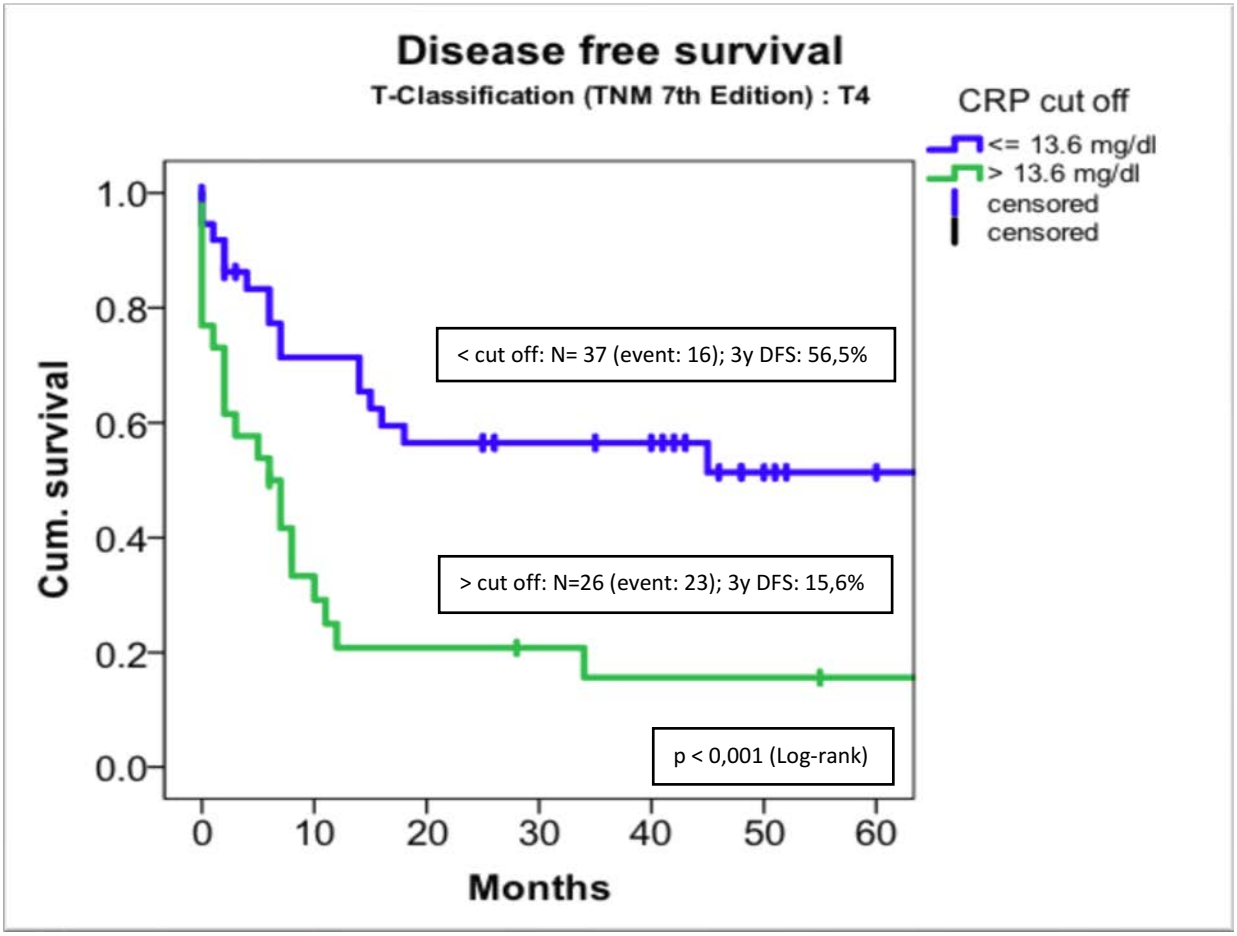


Figure 30: Disease free survival in patients with oropharyngeal cancer: T4 carcinoma

Abbreviation: 3y DFS = 3-year disease-free survival

3.10 Analysis of Fibrinogen and the association with DFS

Using ROC curve analysis, a pretreatment fibrinogen cutoff value of 384 mg/dl was determined (Figure 31). At this cutoff point sensitivity was 0,533, specificity was 0,719 and AUC was 0,681. Fibrinogen levels equal to or below the cutoff point were considered low (n=37/62 patients), while fibrinogen levels above the cutoff point were considered high (n=25/62 patients).

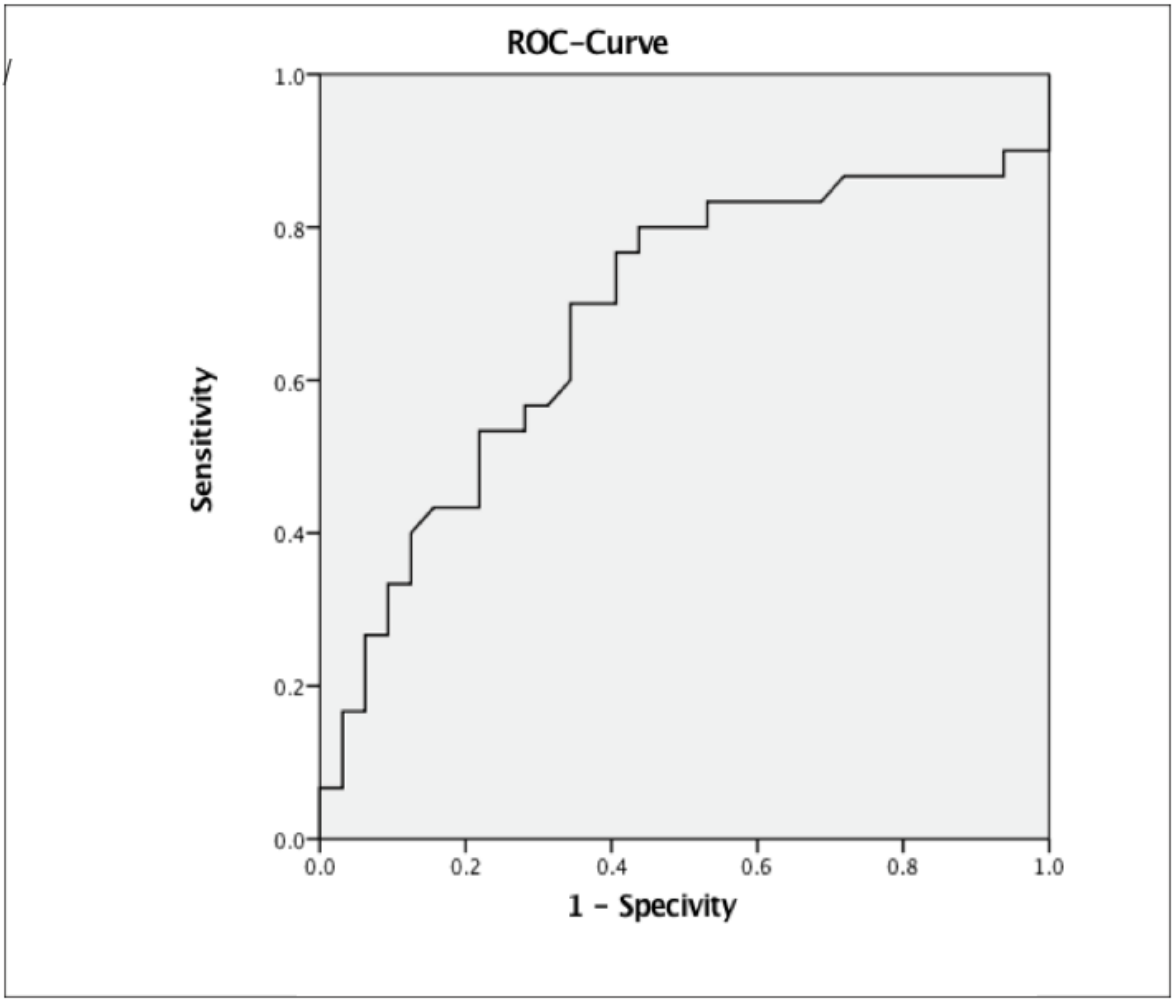


Figure 31: ROC-Curve Firbinogen

The cohort did not show significant differences in DFS in the low fibrinogen vs. the high fibrinogen group (p =0,087) (Figure 31).

In the fibrinogen group <384 mg/dl the mean DFS was 51,9 ± 5,7 months (CI: 40,7 - 63,2). The fibrinogen group >384 mg/dl showed a mean DFS of 37,9 ± 6,7 months (CI: 24,7- 51,2).

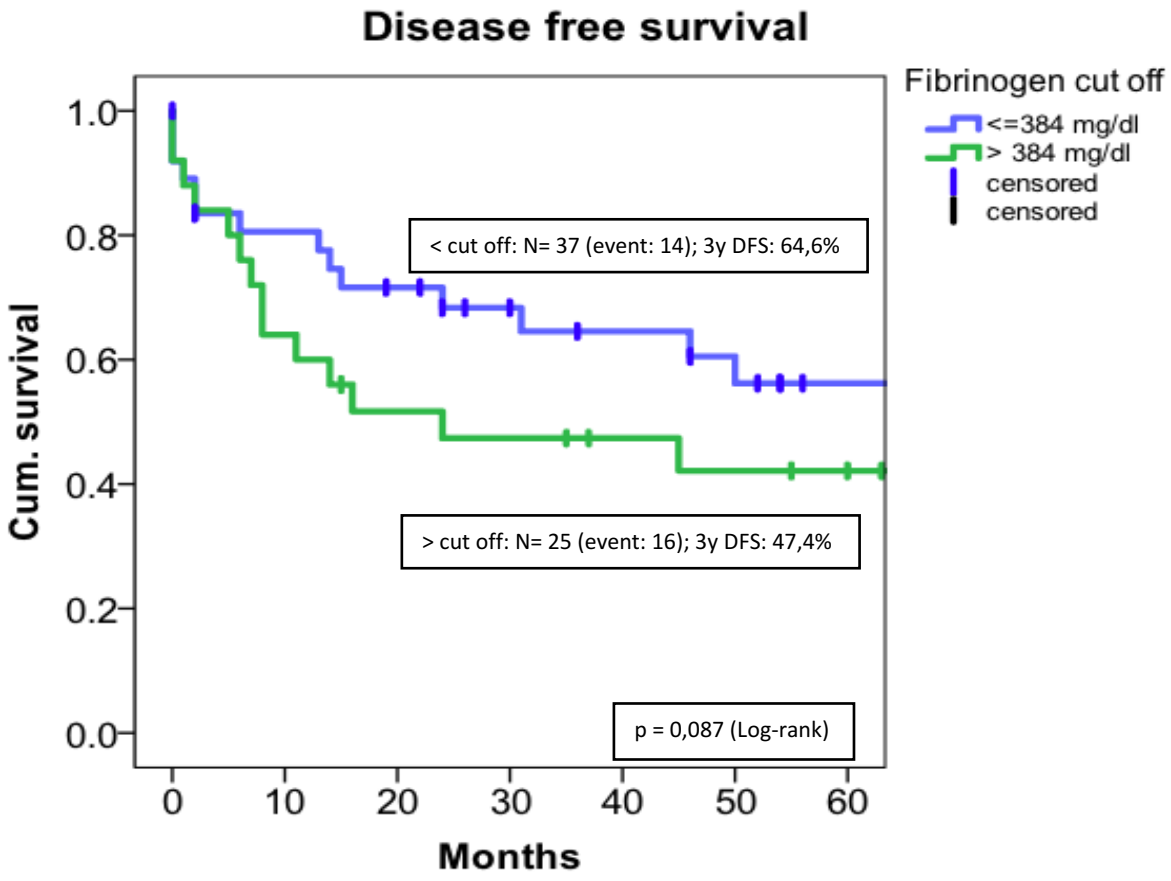


Figure 32: Fibrinogen and DFS in patients with oropharyngeal cancer;

Abbreviation: 3y DFS = 3-year disease-free survival

3.11 Cox regression analysis

In the multivariable analysis, to test whether the variables influence each other or stay independently significant, following variables remained significant (Table 4):
T-classification (p=0,033), AJCC-stage (p=0,025), ECOG-stage (p=0,000) and CRP (p=0,001).

	B	SE	Wald	Df	Significance	Exp(B)
T-Classification (TNM)	0,354	0,166	4,536	1	0,033	1,424
Tumor Localisation	0,060	0,140	0,184	1	0,668	1,062
AJCC	0,570	0,254	5,030	1	0,025	1,769
N-Classification (TNM)	-0,155	0,129	1,441	1	0,230	0,856
ECOG	0,592	0,135	19,303	1	0,000	1,808
CRP	0,026	0,008	11,177	1	0,001	1,026
Leucocytes	0,013	0,063	0,042	1	0,838	1,013
Neutro. G. in %	0,032	0,042	0,587	1	0,443	1,033
Lymphocytes in %	0,018	0,049	0,136	1	0,712	1,018
NLR	-0,001	0,065	0,000	1	0,993	0,999

Table 4: Multivariate analysis

4. Discussion

In 1863, Rudolf Virchow was the first person to describe a correlation between inflammation and cancer. His hypothesis, that chronic inflammation is the origin of cancer, was based on the observation of lymphoreticular infiltrates in neoplastic tissue.[86]

Numerous clinical studies of the past years have demonstrated associations between systematic inflammation and poorer prognosis of cancer patients, using inflammation markers like CRP and fibrinogen.[87] CRP is an acute phase protein, which is primarily synthesized in the liver in response to stimulation by pro-inflammatory cytokines such as interleukin 6 (IL-6). The mechanisms about the relationship between elevated CRP levels and cancer prognosis are still being discussed.[88]

For decision-making in cancer therapy, it is crucial for clinicians to have reliable biomarkers. One such potentially significant marker is CRP, which is easy to measure, repeatable and widely available.

Elevation of pretreatment CRP levels have been reported to be a prognostic indicator in several solid tumors such as renal cell carcinoma[89], gastrointestinal malignancies[90], lung[91] and breast cancer[92], but there are only few studies dealing with oral/oropharyngeal cancer and CRP and the results have been inconsistent.

Thus, the aim of this work is to evaluate the potential of C-reactive protein and pretreatment plasma fibrinogen as prognostic factors in oropharyngeal squamous cell carcinoma.

In this retrospective study 161 patients with oral and oropharyngeal squamous cell carcinoma were included in this analysis, 39 were female (24,2%) and the median age of the cohort at first diagnosis was 61 ± 10.25 years, thus showing no differences with the consulted literature.[93]

The mean follow-up was 31 ± 23.2 months, during which 45 patients died (28%), 37 (82%) cancer related. The mean age at death was 62 ± 10.58 years.

As would be expected from the literature, the most frequent tumor sites were tonsils (n=92 (57,1%)), followed by root of the tongue (n=36 (22,4%)) and oropharyngeal wall (n=33 (20,5%)) (Figure 6).[94] Contrary to expectations, our results did not show a significant association between tumor location and OS/DFS, whereas we observed a highly significant correlation between T-classification and OS/DFS.

In this study the mean value of serum CRP was 17,7 mg/dl; whereas other studies used 5 mg/dl as a cutoff value for CRP, in this study, using ROC curve analysis, a pretreatment CRP cutoff value of 13,6 mg/dl was determined and CRP levels equal to or below the cutoff point were considered low (n=102/143 patients), while CRP levels above the cutoff point were considered high (n=41/143 patients).[95]As CRP is frequently slightly elevated in patients with carcinomas an adjusted cutoff value was of importance.

We divided our cohort in three groups depending on the localization of the tumor. Our results showed that patients presenting with elevated levels of pretreatment CRP and with tumors located in the oropharyngeal wall and in the root of tongue had a significant lower OS (p = 0,023, Figure 21, p = 0,029, Figure 23), whereas in patients with tumors located in the tonsils only a tendency but no significant lower OS was found (p =0,398, Figure 22). However, in the total cohort low levels of CRP show significantly better OS (p =0,003, Figure 20), supporting results of several other studies.[96] In the study "Preoperative circulating C-reactive protein levels predict pathological aggressiveness in oral squamous cell carcinoma: A retrospective clinical study" by Chen et al. preoperative serum CRP levels also were associated with advanced tumor stage, bone invasion, lymph node metastasis and lymph node extra-capsular spread.[97] Hsu et al. showed in the study "Serum markers of CYFRA 21-1 and C-reactive proteins in oral squamous cell carcinoma" that elevated levels of CRP also correlate with skin and bone invasion, tumor depth and pathological tumor status.[98]
Likewise in this study raised CRP in patients with a T3 carcinoma (p =0,04, Figure 24) and T4 carcinoma (p =0,05, Figure 25) showed significant lower OS.
Furthermore, we evaluated the outcome on DFS: In this study elevated levels of pretreatment CRP showed significant lower DFS in the entire cohort (p <0,001, Figure 26) and also in the subgroups; the oropharynx group (p =0,003, Figure 27), the tonsil group (p =0,011, Figure 28) and the root of the tongue group (p =0,039, Figure 29). Moreover, patients with a T4 carcinoma also showed a highly significant lower DFS (p <0,001, Figure 30).
Furthermore, in multivariable analysis (Cox regression), T-classification, ECOG-stage, AJCC-stage and CRP are significant prognostic factors (Table 4).

Thus, we can confirm the results of other studies such as the study “Refining the Role of Preoperative C-Reactive Protein by Neutrophil/ Lymphocyte Ratio in Oral Cavity Squamous Cell Carcinoma” by Fang et al., who in addition to the association between CRP and DFS/OS also showed an association between CRP and pathological tumor status, tumor depth and pathologic nodal metastasis. In the study “Serum C-reactive protein as a prognostic indicator in patients with oral squamous cell carcinoma” Khandavilli et al. showed that raised CRP levels are related to worse OS and described that tumor size and stage when combined with CRP levels increases the predictive power of this indicator.[99]
Huang et al. described in the study “Risk Stratification in Oral Cavity Squamous Cell Carcinoma by Preoperative CRP and SCC Antigen Levels” a significant association between elevated CRP levels and pathologic tumor status, pathologic nodal metastasis, tumor depth, DFS and OS.[100]
Yet, several studies contradict these findings. In the study “C-reactive protein levels: a prognostic marker for patients with head and neck cancer?” by Kruse et al. no association between CRP and development of recurrence and metastases was found, therefore this study does not confirm the results from other studies.[101] However, due to our findings we disagree with Kruse et al..

Nevertheless, there are some limitations that need to be discussed. First, we did not assess potential confounding factors such as local or systemic infections, which could have affected the laboratory data collected on the inflammatory biomarkers. Second, due to its retrospective study design a selection bias in our study cohort cannot be fully excluded.
Third, due to the small number of patients who have been tested for fibrinogen, a significant impact to DFS/OS could not be found. Therefore, further studies have to be performed to validate our findings, especially a prospective study would be of enormous value.

Within the limitations of a retrospective cohort study, we conclude that our data provide strong evidence that CRP, a biomarker which is rapid, minimally invasive and inexpensive, is a useful prognostic tool of disease outcome and treatment response and merits further validation.

5. References

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