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Systemic inflammation and prognostic markers in patients with head and neck cancer

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Abstract

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Patients with head and neck cancer (HNC) often present with weight loss and malnutrition caused by systemic inflammation and dysphagia. This thesis explores the effects of systemic and local inflammation in the context of head and neck cancer treatment.

The main aim of Paper I was to investigate whether trismus after radiotherapy affects the 5-year overall survival rate in a cohort of 244 patients with HNC. The maximum interincisal opening (MIO) of the patients was measured before treatment, 2, 6, and 12 months after the termination of radiotherapy, and trismus was defined as MIO ≤ 35 mm. All patients received instructions on jaw-opening exercises. The highest prevalence of trismus at 12 months was seen in patients with oral cancer (44%) and oropharyngeal cancer (37%) and it can be concluded that these patients should primarily be offered jaw-opening exercises. Patients with trismus at 12 months after termination of treatment had a tendency towards a worse overall 5-year survival rate than patients without trismus (p=0.64).

Paper II explored the expression levels of cytokines and growth factors in serum before and up to one year after treatment. The cohort consisted of 30 patients with HNC and blood was drawn on four occasions and analyzed for 10 cytokines and 4 growth factors. Patients who received chemoradiotherapy had higher expression levels of IL-1β, IL-6, and IL-10 than other treatment groups at 7 weeks after the start of treatment. Patients with recurrence within 12 months after termination of treatment had higher expression levels of IL-1β, IL-6, IL-8, and IL-10 than the remaining patients at 7 weeks.

In Paper III the expression levels of 83 immuno-oncologically significant proteins were determined at the same four time points as in Paper II in a cohort of 180 patients with HNC using a proteomics technique. Fifteen proteins had either decreased or increased expression levels at 7 weeks compared to pre-treatment expression levels. Treatment with radiotherapy with concomitant cisplatin was shown to be connected to significantly decreased expression of 13 proteins at 7 weeks after the start of treatment compared to radiotherapy which demonstrates an immunomodulatory effect of cisplatin also apparent in Paper II.

The aim of Paper IV was to investigate how pre-treatment body mass index and fat free mass index correlate with early death in a cohort of 404 patients with head and neck squamous cell carcinoma (HNSCC). Body mass composition was measured at diagnosis using bioelectrical impedance analysis. Patients who died within 6 months of the start of treatment had significantly lower FFMI at diagnosis compared with patients who survived beyond 6 months (17.6 kg/m2 and 19.5 kg/m2, respectively, p=0.035). It can be recommended that assessment of fat free mass index should be included in the management of patients with HNSCC prior to treatment.

Keywords: trismus, body mass composition, radiotherapy, cisplatin, proteomics

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To Benedikt, Júlía and Kristján

Cover photo by Ragnar Th. Sigurdsson,
arctic landscape photographer and oropharyngeal cancer survivor

List of Papers

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Thorsteinn Astradsson, Göran Laurell, Alexander Ahlberg, Polymnia Nikolaidis, Hemming Johansson, Ylva Tiblom Ehrsson. Trismus in patients with head and neck cancer and 5-year overall survival. Acta Otolaryngol. 2018 Dec;138(12):1123-1127. Doi: 10.1080/00016489.2018.1511059

II. Thorsteinn Astradsson, Felix Sellberg, David Berglund, Ylva Tiblom Ehrsson, Göran Laurell. Systemic inflammatory reaction in patients with head and neck cancer – An explorative study. Front Oncol. 2019 Nov 5;9:1177. Doi: 10.3389/fonc.2019.01177.

III. Thorsteinn Astradsson, Felix Sellberg, Ylva Tiblom Ehrsson, Karl Sandström, Göran Laurell. Serum proteomics in patients with head and neck cancer: Peripheral blood immune reaction to treatment and recurrence. Int J Mol Sci. 2022 Jun 4; 23(11):6304. Doi: 10.3390/ijms23116304.

IV. Charbél Talani[¤], Thorsteinn Astradsson[¤], Lovisa Farnebo, Antti Mäkitie, Ylva Tiblom Ehrsson* and Göran Laurell*. Pretreatment fat-free mass index correlates with early death in patients with head and neck squamous cell carcinoma. Head & Neck 2024 Apr;46(4):808–818. Doi: 10.1002/hed.27628.
[¤] Shared co-first authorship
*Shared last authorship

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Contents

Acronyms

ARG1	Arginase 1
BIA	Bioelectrical impedance analysis
BMC	Body mass composition
BMI	Body mass index
CCL20	CC motif chemokine ligand 20
CCL22	CC motif chemokine ligand 22
CCL17	CC motif chemokine ligand 17
CXCL9	CXC motif chemokine ligand 9
CXCL10	CXC motif chemokine ligand 10
CRT	Chemoradiotherapy
CT	Computed tomography
CTLA-4	Cytotoxic T lymphocyte antigen 4
CUP	Cancer of unknown primary
DAMPs	Damage-associated molecular patterns
DC	Dendritic cell
DEXA	Dual-energy X-ray absorptiometry
DOI	Depth of invasion
EBV	Epstein-Barr virus
ECOG/WHO	Eastern Cooperative Oncology Group/World Health Organization
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FASL	Fas ligand
FDG-PET	Fluorodeoxyglucose positron emission tomography
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FFM	Fat-free mass
FFMI	Fat-free mass index
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
GLIM	Global Leadership Initiative on Malnutrition
GM-CSF	Granulocyte-macrophage-colony-stimulating factor
Gy	Gray

HRQoL	Health-related quality of life
HNC	Head and neck cancer
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papillomavirus
ICI	Immune checkpoint inhibitor
irAEs	Immune-related adverse events
IMRT	Intensity modulated radiotherapy
IFN-γ	Interferon-γ
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-12	Interleukin-12
MHC	Major histocompatibility complex
MIO	Maximum interincisal opening
MMP20	Matrix metalloproteinase-20
MRI	Magnetic resonance imaging
NPX	Normalized protein expression
OCC	Oral cavity cancer
OPC	Oropharyngeal cancer
PD-1	Programmed death 1
PD-L1	Programmed death ligand-1
PEA	Proximity extension assay
PEG	Percutaneous endoscopic gastrostomy
RIG	Radiologically inserted gastrostomy
RFS	Radiation fibrosis syndrome
RMO	Reduced mouth opening
RT	Radiotherapy
SCC	Squamous cell carcinoma
SMM	Skeletal muscle mass
SNB	Sentinel lymph node biopsy
SNUC	Sinonasal undifferentiated cancer
3DCRT	Three-dimensional conformal radiotherapy
TAM	Tumor-associated macrophages
TCR	T-cell receptor
TMJ	Temporomandibular joint
TME	Tumor microenvironment
TNF-α	Tumor necrosis factor α
TORS	Transoral robotic surgery
VEGF	Vascular endothelial growth factor
VMAT	Volumetric modulated arc therapy

Introduction

Epidemiology

Head and neck cancer (HNC) is the sixth most common type of cancer with an estimated 930,000 new cases annually worldwide[1]. According to the Swedish Head and Neck Cancer Register (SweHNCR), which was established in 2008, approximately 1700 patients are diagnosed with HNC each year in Sweden with a general preponderance of males and a median age ranging from just below 60 years to 76 years, depending on tumor location[2]. The incidence of HNC in Sweden has significantly increased from when the register was established, which is mainly attributable to the increase in human papillomavirus (HPV) positive oropharyngeal cancer (OPC), which is a worldwide phenomenon, especially in high-income countries[3].

Around 70% of patients diagnosed with HNC have either oral cavity cancer (OCC), OPC or laryngeal cancer[1]. The remaining tumor locations included in HNC are sinonasal cancer, salivary gland cancer, lip cancer, nasopharyngeal cancer, hypopharyngeal cancer and cancer with unknown primary (CUP). There are geographical variations, most notably in the case of nasopharyngeal cancer, which has a much higher incidence in Southeast Asia compared to other parts of the world, including Sweden, where around 30 cases are diagnosed each year[2]. The classic risk factors for developing HNC are excessive alcohol consumption and smoking but, as mentioned above, infection with HPV virus has emerged as the main driving force for the increase in the incidence of OPC in recent decades. The high incidence of OCC is expected to increase in India and Southeast Asia[4]. The main oncogenic virus types are HPV 16 and 18, along with HPV 33. Other important risk factors include the consumption of betel nuts, which greatly increases the risk of OCC, and the Epstein-Barr virus (EBV), which can cause nasopharyngeal cancer[5,6]. Also, like many other types of cancer, obesity has been linked to an increased risk of HNC[7,8]. Moist oral snuff (snus) has not been shown to be associated with the risk of developing OCC[9]. The use of electronic cigarettes has increased in recent years and time will tell whether this will affect the incidence of HNC[10]. There is a general preponderance of males in HNC, especially in laryngeal and hypopharyngeal cancer[2,11]. Little or no gender difference is seen in OCC and salivary gland malignancies[2]. The most common histology seen in HNC is squamous cell carcinoma (90%), i.e., head and neck squamous cell carcinoma, HNSCC, which dominates in OCC, OPC, laryngeal cancer, lip cancer and hypopharyngeal cancer. In other tumor locations, a different histology is encountered more frequently, including adenoid cystic cancer, mucoepidermoid cancer of the salivary glands and adenocarcinoma. Salivary gland cancer histopathology has indeed been expanded recently in the 5th edition of the World Health Organization Classification of Head and Neck Tumours, which now includes 21 different salivary gland malignancies[12].

Diagnosis

Patients present with different signs and symptoms of HNC depending on the tumor location. A hoarse voice in a patient with a history of smoking can indicate glottic laryngeal cancer, while dysphagia might be the predominant symptom in a patient with supraglottic laryngeal cancer or hypopharyngeal cancer. Patients with OPC typically present with neck metastases in addition to local throat symptoms, while OCC can become apparent when a wound in the oral cavity fails to heal. Weight loss and sarcopenia are common features at diagnosis of HNC, especially in patients presenting in an advanced stage[13]. This can be attributed to dysphagia due to the tumor disease and a systemic inflammation commonly caused by the cancer[14,15]. However, in its early stages, HNC may not cause any symptoms, thereby increasing the risk of delayed diagnosis.

The diagnosis of HNC is based on clinical examination, biopsy and various radiological investigations. In OCC a biopsy is most often easily attained, while a laryngeal biopsy usually requires general anesthesia. Fine needle aspiration cytology is carried out on suspected lymph node metastasis and even core needle biopsy when a larger sample is needed in order to achieve a definitive diagnosis. Open biopsies of lymph nodes for diagnosis should be avoided because of the risk of tumor spillage. Apart from a general histopathological diagnosis, including immunohistochemistry such as p16 (an HPV surrogate marker), further analyses may be required, such as HPV and EBV detection, through in-situ hybridization or polymerase chain reaction[16]. Furthermore, programmed death ligand-1 (PD-L1) immunostaining can be performed to determine the feasibility of treatment with immune checkpoint inhibitors (ICI)[17]. In recent years, sentinel lymph node biopsy (SNB) has become important in the diagnosis and staging of early-stage OCC, where a negative biopsy can spare the patient from undergoing an elective staging neck dissection[18]. Most patients require a computed tomography (CT) investigation of the neck and thoracic cavity for evaluation of the extent of the primary tumor, as well as regional and distal metastasis. Magnetic resonance imaging (MRI) can be helpful in many cases such as for evaluating cartilage invasion in laryngeal cancer. Fluorodeoxyglucose (FDG) positron emission tomography (FDG-

PET/CT) has also become important, especially for follow-up after radiotherapy (RT) or chemoradiotherapy (CRT) for OPC, but can also be used in the diagnostic phase to exclude generalized disease and detect an occult primary tumor[19]. The use of MRI in combination with FDG-PET will likely become a prominent mode of investigation in the future[19].

Delay from the onset of symptoms to diagnosis is rather common in HNC with significant contributions from both patient delay and provider delay[20]. Between 50–70% of patients present in an advanced stage (III–IV)[21,22]. It is therefore of great importance to reduce the delay of diagnosis, but also any delay that can occur from time of diagnosis to the start of treatment as this can also affect the outcome and survival rate[23,24]. In Sweden, a standardized care pathway was implemented in 2015 for various groups of cancer in order to streamline both the referral and diagnostic process and reduce the waiting times for treatment[25]. HNC was included in this standardized care pathway (standardiserat vårdförlopp, SVF) and this has likely reduced the lag time from symptom onset to the start of treatment.

All patients with HNC should be staged according to the TNM classification system, which is used to document the anatomical extent of the cancer and is of great importance for predicting prognosis and determining treatment recommendations. The classification can be either clinical or pathological. The general rule is that tumors confined to the organ of origin are classified as stage I and II while more extensive locoregional spread is classified as stage III or IV, and distant metastasis is classified as stage IV[26]. The 8th and most recent version of this classification system differentiates between HPV-positive and HPV-negative OPC, which is important as the former carries a much better prognosis and is now still regarded as stage I or II even in the presence of regional neck metastasis[27]. In addition, depth of invasion (DOI) has been introduced as a variable in the staging of OCC, where a DOI of more than 5 mm will advance the primary tumor from T1 to T2, or T3 if the DOI exceeds 10 mm[27]. An example of an OCC situated in the right bucca is shown in figure 1.

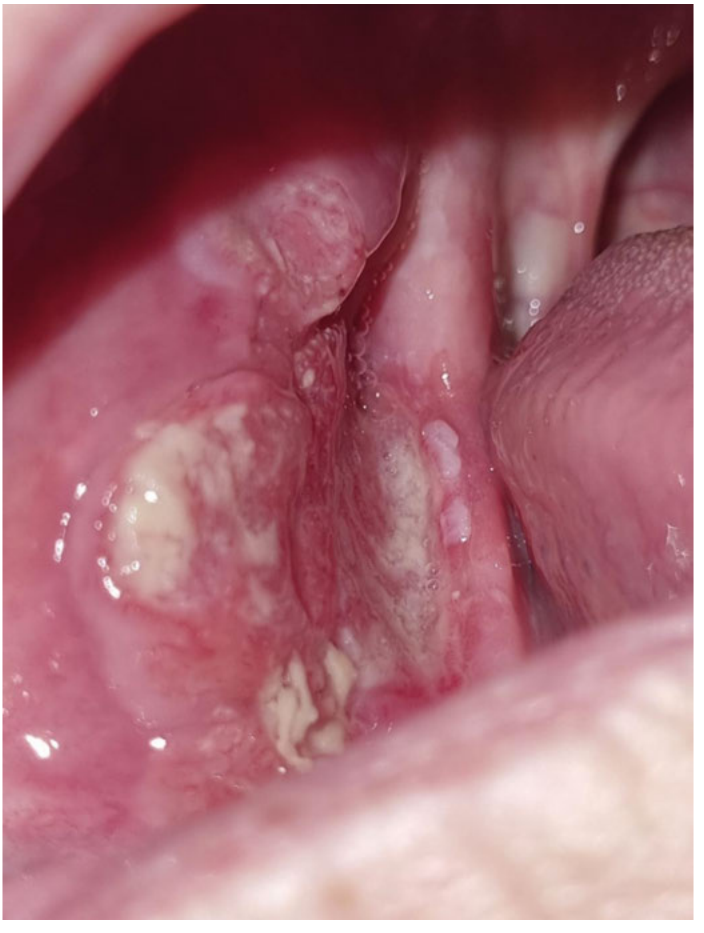

Figure 1 - Buccal cancer. Published with patient consent.

Treatment

The treatment plan for patients with HNC should be discussed in a multidisciplinary setting in which the most crucial decision is whether the patient should be offered treatment with curable intent or palliation and the best supportive care. A recent survey showed that 90% of all HNC patients in the Nordic countries are discussed at a multidisciplinary meeting[28]. In Sweden, all patients with HNC, apart from patients with small lip cancer (T1N0M0), are discussed in such a setting[29]. The general performance status of the patient should be assessed before any treatment recommendation is offered, using recognized tools such as the Eastern Cooperative Oncology Group (ECOG)/World Health Organization (WHO) performance scale[30]. After being carefully informed about all treatment options, the patient should have the final say on which treatment they accept.

Radiotherapy

Conventional RT uses photons and is the cornerstone of HNC treatment[31]. The toxicity of RT is considerable although the evolution of intensity-modulated RT (IMRT) and volumetric modulated arc therapy (VMAT) has very likely reduced the side effects. However, very little data exist that have compared these techniques to previous techniques, at least not in terms of very late complications and tumor control[32,33]. IMRT is an advanced version of a previous technique, 3D conformal radiotherapy (3DCRT), and uses so-called inverse treatment planning in which the maximum dose is delivered to the areas that are in greatest need while sparing the healthy surrounding tissue and organs at risk[34]. VMAT can be considered a variation of IMRT but delivers the radiation with a rotating arc instead of a fixed field[35].

There are other kinds of RT, both local intratumoral radiation therapy (brachytherapy) and proton radiation. Currently, the latter is primarily used in the re-irradiation setting, though it will likely have a larger role in the treatment of HNC in the future[36]. At present, the Skandion clinic in Uppsala is the only center in Sweden to offer proton therapy.

HNSCC is described as moderately radiosensitive and fairly high doses are required to achieve a cure, a total dose of 68–70 Gray (Gy) is usual[37,38]. In Sweden, the standard fractionation is 2 Gy per day for 5 days a week[29]. For neoadjuvant/pre-operative RT, the dose can range from 46–70 Gy, while for adjuvant/post-operative RT, the total dose is usually over 60 Gy[29].

As p16 positive/HPV-positive OPC is considered more radiosensitive than p16 negative/HPV-negative OPC and carries a better prognosis, de-escalation of treatment for this patient group has been widely studied[39]. However, no consensus has been reached regarding this[40].

Chemotherapy

Cisplatin is an alkylating drug and for many years has been the chemotherapeutic agent of choice in HNC. Evidence indicates that in addition to its conventional cytotoxic effects, it also has an immunomodulatory effect[41,42]. The main side effects related to cisplatin treatment are nephrotoxicity and ototoxicity along with hepatotoxicity[43].

As a part of curative treatment, indications for chemotherapy include concurrent treatment with RT in nasopharyngeal cancer, hypopharyngeal cancer and OPC. The usual dose range for concomitant cisplatin is 30–40 mg/m^2/week up to a minimum dose of 200 mg/m^2 in total. There might be an indication for induction chemotherapy as well in the treatment of some locally advanced tumors such as laryngeal cancer in order to increase the likelihood of organ preservation, as well as in nasopharyngeal cancer, but this has been the subject of debate[44,45].

Targeted therapy

Targeted therapy refers to treatment that targets specific genes or molecules to exert an anti-tumor effect. Targeted therapy agents are either small molecule drugs or monoclonal antibodies. Cetuximab, an epidermal growth factor receptor (EGFR) inhibitor, is currently the only targeted therapy agent approved for the treatment of HNC[46]. Previous studies on locally advanced HNSCC have demonstrated the benefit of concurrent cetuximab therapy with RT compared to RT only[47,48]. However, cetuximab has been shown to be inferior to cisplatin in a concurrent treatment setting and is currently only considered an alternative when cisplatin is contraindicated[49].

Immunotherapy

In recent years, immunotherapy has revolutionized the treatment of various forms of cancer, most notably the treatment of malignant melanoma and lung cancer. The ICIs are at the core of immunotherapy and block either cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1) or PD-L1[50]. At present, immunotherapy with ICIs is considered a first-line treatment in recurrent or metastatic HNC, either as monotherapy or in combination with platinum-based compounds[29,51]. Multiple studies are ongoing and immunotherapy will likely play a more prominent role in the primary treatment of HNC in the future, including as a neoadjuvant agent before surgery[52,53].

Treatment by location

For patients with early-stage disease, a single modality treatment is often sufficient for cure. For smaller, localized OCC lesions, for example, an excision with an adequate surgical margin will often be the only treatment needed for the primary tumor. Similarly, RT can provide a cure for patients with early-stage OPC[54]. An alternative to RT in selected OPC cases is transoral robotic surgery (TORS)[55].

As previously mentioned, the majority of patients with HNC are diagnosed at a more advanced stage and require multimodality treatment consisting of some combination of surgery, RT or CRT.

Advanced stage OCC, if deemed curable, is typically treated with surgery of the primary tumor with elective or therapeutic neck dissection followed by adjuvant RT or CRT with cisplatin. The defect created by the excision of the primary tumor frequently requires reconstruction with a microvascular-free flap, either a soft tissue flap or a bone flap if a defect in the mandible or maxilla is to be reconstructed. The field of head and neck microvascular surgery has expanded greatly over the past three to four decades allowing for complex and large tumor resections to be performed with excellent reconstructions that are more acceptable, both functionally and aesthetically, than older pedicled flap techniques[56].

More advanced OPC is usually treated with CRT, irrespective of HPV status[57]. Due to the high risk of lymph node metastasis, the N0 neck also needs to be treated with radiation or neck dissection, even for early-stage OPC[58].

The treatment of laryngeal cancer largely depends on subsite and stage. In recent decades there has been a focus on organ preservation techniques[59]. Early-stage glottic tumors can often be excised using transoral laser microsurgery, but RT is considered equal in terms of survival and voice quality[60,61]. However, more advanced tumors (T3) require RT or CRT[60]. Tumors in the supraglottic area have traditionally been treated with RT or CRT but partial laryngectomies have become an alternative form of treatment in a number of centers for both glottic and supraglottic tumors[62]. In the case of supraglottic tumors, the neck needs to be addressed irrespective of which treatment modality is chosen[62]. Total laryngectomy remains an important and effective primary treatment option for T4a tumors, as well as in a salvage situation[63].

Hypopharyngeal cancer patients often present in an advanced stage and the treatment of choice if the patient is still in a curable stage is most often CRT[64]. Endoscopic or robot-assisted resection can be considered for small tumors while a pharyngolaryngectomy with reconstruction is reserved for patients in good general health with locally advanced tumors or, more commonly, in a salvage situation[65,66].

Sinonasal tumors typically require both primary surgery if resectable and adjuvant therapy[29]. As this tumor location is rather heterogeneous in nature, there are some exceptions, such as sinonasal undifferentiated cancer (SNUC), for which the appropriate treatment is induction chemotherapy followed by CRT if a treatment response is noted[67].

The treatment of salivary gland cancer should consist of primary surgery with or without neck dissection[68]. The decision to offer postoperative RT depends on a number of risk factors including the histological type and grade and the presence of perineural invasion[68,69].

The majority of patients with nasopharyngeal cancer receive combination treatment in which RT is the cornerstone and chemotherapy is given either concomitantly and/or as an induction therapy[29,70]. Considering the close proximity of this area to sensitive organs such as the eyes, brain and pituitary gland, precise treatment planning is needed to reduce the risk of serious acute and long-term complications.

Surveillance and support strategies

Follow-up after therapy for HNC is key to the early detection of recurrence, second primary tumors and managing the side effects of treatment. In general, clinical follow-up is scheduled at three-month intervals in the first two years after treatment and then at six-month intervals for an additional three years[29]. An exception to this is adenoid cystic cancer, for which follow-up should exceed 10–15 years due to its indolent course and tendency for late recurrence[71]. The indications for post-treatment radiological surveillance vary between tumor sites. The use of FDG-PET is now widespread for the assessment of treatment response in OPC, in which the optimal timing is considered to be 10–12 weeks after end of treatment[72-74]. MRI is a useful tool for evaluating tumor regression in both the nasopharynx and sinonasal region and also CT for the detection of lung metastases. Generally, routine radiological investigations are not recommended without a specific clinical indication after the initial post-treatment evaluation.

The need for rehabilitation should be assessed at an early stage, preferably before the initiation of treatment. In general, the rehabilitation needs of cancer patients are often unmet and HNC is no exception[75,76]. This applies to both physical rehabilitation and also psychological and sexual rehabilitation, and includes social and financial guidance[75]. The term *prehabilitation* has gained popularity in the past decade and, as the name implies, is aimed at optimizing the patient's overall physical and psychological well-being before treatment. However, in a number of studies, prehabilitation has been described to continuing concurrently with treatment prior to the onset of symptoms of dysfunction[77,78]. Although the effectiveness of prehabilitation before treatment is yet to be conclusively proven, in theory it might reduce the need for rehabilitation and soften the impact of disease-related and treatment-related morbidity, for example, dysphagia and trismus in HNC.

Patients with HNC are at great risk of malnutrition as dysphagia is a common presenting symptom, leading to reduced calorie intake, which exacerbates the effect of cancer-related systemic inflammation and catabolism. The treatment often results in swallowing difficulties. Several studies have shown that malnutrition affects the survival rate in HNC, in both the short term and the long term[79-81]. Monitoring of body weight and even body mass composition is critical during and after treatment. Before treatment, patients should be assessed by both a dietitian and a speech pathologist. Validated screening tools for evaluating swallowing ability and nutritional status should be used[82]. An energy goal of 30 kilocalories/kg/day is recommended and if not met, nutritional drinks and supplements should be added to the diet[83]. Up to 40–50% of patients with advanced stage disease will need enteral nutrition during RT[84]. This can be achieved through a nasogastric feeding tube or percutaneous endoscopic gastrostomy (PEG) if a prolonged period of enteral nutrition is expected. Alternatively, a radiologically inserted gastrostomy (RIG) can be performed. How aggressively one should treat established or expected malnutrition has been the subject of debate. Some caregivers advocate enteric nutrition from the start with prophylactic PEG insertion before treatment, while other caregivers choose to monitor the patient and intervene if their caloric intake deteriorates, using a nasogastric feeding tube or PEG if the period of enteral nutrition is likely to exceed more than 3–4 weeks. The literature does not unequivocally support either approach with regards to survival or quality of life (QoL), but there seems to be a tendency toward a more reactive approach, at least in the Nordic countries[85,86]. According to British guidelines, enteral nutrition should start if energy intake under 60% of the estimated energy expenditure is expected for more than 10 days[82].

Monitoring of mouth opening should be conducted during and after RT or surgery that predisposes the masticatory apparatus to inflammation and fibrosis. Jaw opening exercises can prevent the development of trismus, but the literature has not been clear with regards to the selection of patients for this type of intervention.

Outcomes

The treatment outcome and survival rate in HNC vary greatly between tumor locations and stages and early diagnosis is of utmost importance in most cases of HNC. However, the majority of patients present in an advanced stage (stages III or IV) with large primary tumors and often nodal involvement. However, distant metastases are relatively uncommon at the time of diagnosis but up to 15% of HNC patients will develop spread disease which will most likely occur within two years of primary treatment[87,88]. Cancer recurrence after primary treatment, either locoregionally or distant, carries a poor prognosis in many cases[89].

According to Swedish data, lip cancer has a 5-year survival rate of more than 90% while HPV-positive OPC has a 5-year survival rate of around 80%, compared to less than 40% for HPV-negative OPC[2]. Hypopharyngeal cancer, however, carries the worst prognosis with a 5-year survival rate below 30%[2]. These survival rates are comparable to international reports[90]. A more advanced stage of disease irrespective of tumor location logically confers a worse prognosis in general.

A limited number of studies have been performed on early mortality in HNC. However, in a nationwide register-based study, Talani et al. showed that a higher WHO performance score, advanced disease stage, higher age, hypopharynx location and palliative treatment intent were all predictors of death within 6 months of diagnosis[22]. In this study of almost 10,000 patients, close to 10% did not survive beyond 6 months of diagnosis. Another recent register-based study found that death within 30 days of initiation of definitive RT occurred in 3.5% of the cohort with similar risk factors as the previous study[91].

Health-related quality of life (HRQoL) is often significantly impacted for patients who have had HNC, both in the short term and, in some cases, permanently. These negative effects are not universal, however, and there are individual variations in which a high proportion of HNC patients live their post-treatment lives with little or no sequelae[92]. The negative aspects of treatment that can affect HRQoL vary and range from xerostomia and chronic pain to severe dysphagia and cosmetic disfigurement[93]. This issue should not be ignored, both when giving treatment recommendations, as well as in the aftermath of treatment[94-96]. The term *survivorship* has become prominent in recent years and pertains to the overall well-being of cancer survivors faced with a new reality after the treatment and complications that may subsequently arise[97].

Side effects of treatment

The treatment of HNC is often robust and has the potential to generate significant side effects and complications, especially when patients undergo combined modality treatment. Some side effects may even prove to be life threatening, both in the acute phase and in the long term. The occurrence and degree of side effects have a tendency to show interindividual differences, both predictable and idiosyncratic.

Surgery

The extent of surgery varies according to stage and location, meaning the side-effects of surgical interventions also vary. In most cases, the risk of complications and permanent sequelae such as nerve damage is greater in salvage surgery, when the field has been previously irradiated due to fibrosis and loss of normal planes of dissection[98,99]. The formation of pharyngocutaneous fisutulas after salvage laryngectomy has an incidence of up to 35% and is significantly more common than after primary laryngectomy. However, augmenting wound healing with a pectoralis major flap reduces the risk of this difficult complication[100,101]. Extensive head and neck surgery often causes both cosmetic and functional sequelae, which can greatly affect the individual. External and internal lymphedema is very common and is present in some form in over 90% of patients, especially after multimodal treatment[102,103]. This can result in cosmetic issues and can contribute to dysphagia and poor voice quality[104].

Radiotherapy

Radiation is the cornerstone of HNC treatment and it is estimated that 80% of all HNC patients receive RT during the course of their treatment[105]. A systemic and local inflammatory response is initiated as soon as tissue is radiated and can lead to adverse effects and toxicities[106-108]. The effects of RT, both wanted and unwanted, can be seen outside the irradiated field as a result of this widespread immune activation[109].

The acute adverse effects that most patients experience include mucositis and dysphagia, as well as skin erythema and dermatitis. These effects tend to wane during the weeks following the termination of RT. Mucositis should be assessed using a validated scale such as the WHO Oral Mucositis Grading Scale[110]. The muscle wasting and weight loss that is already established in many patients before treatment is further exacerbated by the inflammation caused by RT and in a number of patients can persist long after the treatment is over[15,111].

Late complications include xerostomia, dysgeusia, and trismus, all of which can affect HRQoL. Xerostomia has a negative effect on dental health and patients should be closely monitored for caries by dental professionals after RT. Osteoradionecrosis is a serious complication that can appear many years after the termination of treatment. The risk of osteoradionecrosis can be reduced with a thorough dental inspection prior to RT[112]. Hypothyroidism is present in the neck of up to 40% of patients after RT and should be screened for[113]. RT of the neck increases the risk of developing carotid stenosis to such an extent that annual Doppler screening following cancer treatment might be indicated[114,115]. Last but not least is the risk of developing a second primary cancer in the irradiated field, mainly squamous cell carcinoma (SCC) or sarcoma[116,117]. The incidence rates reported in the literature vary widely. However, according to a recent publication on a large cohort, the incidence was 1.55 per 100 patient years[118]. The same publication observed no difference in the risk of second primary cancer in a comparison of 3DCRT and IMRT[118]. However, proton beam RT was less likely than the other two modalities of RT to cause a second primary cancer.

Chemotherapy

Cisplatin has been the chemotherapeutic agent of choice in HNC therapy for more than four decades. This alkylating agent has the potential for specific adverse effects, with nephrotoxicity being the most common, and is reported to occur in up to 30% of patients[119,120]. Cisplatin has a neurotoxic potential, which can be reflected in peripheral neuropathy and ototoxicity, both of which are relatively frequent findings after treatment[121,122].

Immunotherapy

ICIs that have become widely used in the treatment of various forms of cancer have a unique adverse effects profile compared to conventional chemotherapy, collectively known as *immune-related adverse events* (irAEs). The immunological checkpoints inhibited by this treatment are central to self-tolerance and the manifestations of toxicity may include deterioration of autoimmune diseases, colitis, pneumonitis and skin rash, although virtually every organ could be affected[123]. PD-1 inhibitors have been shown to have a milder irAE profile than CTLA-4 inhibitors and could therefore be more suitable for patients with autoimmune diseases[124].

Trismus

Reduced mouth opening (RMO) or trismus is a common condition in patients with HNC and is often a manifestation of radiation fibrosis syndrome (RFS). Incidence rates reported in the literature vary greatly, which is probably attributable to the different definitions of trismus, as well as the time points of measurement and the heterogeneity of the patient groups studied. Incidence rates as high as 79% have been reported, although a more realistic incidence rate is probably between 30–50% 6–12 months after treatment[125-128].

In severe cases, trismus can lead to significantly reduced HRQoL, social inhibition and even malnutrition[129-131]. In addition, for patients with trismus, dental hygiene can be challenging, and this also applies to oncological control of the oral cavity and oropharynx, which can lead to delayed diagnosis of tumor recurrence and second primaries. The relationship between trismus and survival in HNC has been poorly investigated in the literature but it is plausible to assume that severe trismus following treatment can be associated with a worse overall prognosis, for example, through malnutrition, aspiration, increased risk of recurrence and delay in the diagnosis of recurrence.

Mouth opening is determined by measuring the interincisal opening distance or the distance between the alveolar ridges in edentulous patients. A cutoff of 35 mm is now widely accepted as the threshold of trismus in head and neck oncology, i.e., a maximum interincisal opening (MIO) of 35 mm or below should be defined as trismus[132]. The muscles of mastication responsible for closing the mouth are the masseter, medial pterygoid and temporal muscles, while the lateral pterygoid muscle opens the mouth with help from the mylohyoid muscle.

In the context of HNC, trismus is most often caused by RT but in the case of OCC, OPC, parotid gland cancer, nasopharyngeal cancer and sinonasal cancer, trismus can be caused by direct tumor invasion of the muscles of mastication and surrounding soft tissues, as well as the temporomandibular joint (TMJ). Tumors in the infratemporal fossa or the parapharyngeal space can also present with trismus as the sole symptom. This can contribute to the dysphagia and malnutrition that many patients exhibit at the time of diagnosis. The treatment then exacerbates the trismus, RT in particular, which initiates a process that is thought to begin with hypoxia and thrombosis of small vessels[133,134]. This leads to an influx of neutrophils and other mediators of inflammation[135]. This chronic inflammation or pre-fibrotic phase eventually evolves into fibrosis formation that is characterized by the presence of myofibroblasts in the affected tissue in which the microvascular supply gradually becomes weaker [133,136]. Denervation also contributes to the development of trismus and to reduced power and/or spasms of the muscles of mastication[133,137]. In the final and permanent phase, atrophy of normal tissue will have occurred, and the fibrosis becomes established.

The development of trismus can be halted or reversed with mouth opening exercises and various instruments have been developed for this, such as Therabite® and Acute Medic Jaw Trainer and Stretcher® shown in Figure 2. Significant compliance is needed for mouth opening exercises to be effective and the selection of patients and timing of exercises are still unclear, although it can be assumed that "the earlier the better" applies here. However, a recent study demonstrated improvements in MIO whereby exercises were introduced on average 6.2 years post-treatment, indicating that late intervention could also be effective[138]. Surgical treatment is indicated in selected cases, for example, resection of obvious fibrous bands in the bucca, or coronoidectomy[139]. Medical treatment has not been proven to be useful in studies on pentoxifylline and hyperbaric oxygen, but botulinum toxin injections can reduce the muscle spasms and pain involved in the pathogenesis of trismus, but likely have no direct effect on mouth opening[140-142].

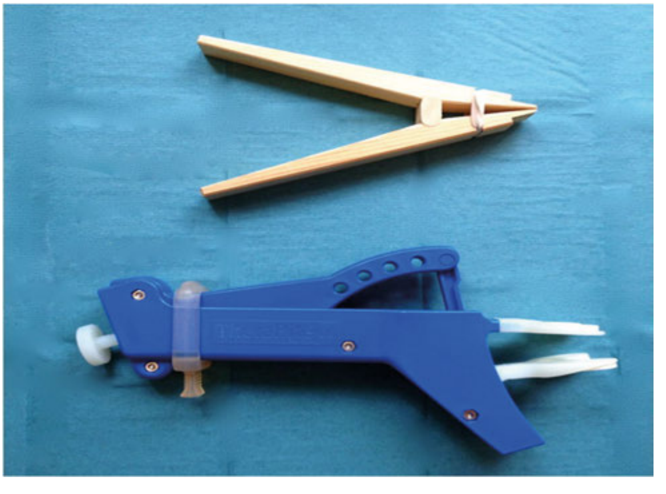

Figure 2 - Acute Medic Jaw Trainer and Stretcher (above) and Therabite (below). Reproduced from Karlsson et al. (Springer)[143].

Immune response, proteomics and biomarkers

Immune response

It has become increasingly clear that the immune system is instrumental in the prevention, response to treatment and progression of cancer. This is reflected in the fact that inflammation is now considered one of the hallmarks of cancer and chronic inflammation, along with infection, is estimated to be the major driver for 25% of all cancer cases [144,145]. Even though our understanding of the processes involved has come a long way since Virchow identified leukocytes in tumor specimens more than 160 years ago, there is still much to learn[146].

In immunocompetent individuals, the innate immune system provides continuous monitoring against neoplasia and identifies antigens that are foreign to it. The adaptive immune system is then crucial once a cancer is established. The mechanisms involved in anti-cancer immunity have been well documented by Chen and Mellman and referred to as the *cancer-immunity cycle*[147,148]. This 7-step process begins with tumor neoantigens being exposed to dendritic cells (DCs) which, under the influence of pro-inflammatory cytokines, present the neoantigens to T cells. This results in the priming and activation of effector T cells in lymph nodes. The consequential direction of the immune response that follows depends on multiple factors in which the balance of T effector cells ($CD8^+$) versus T regulatory cells is critical[147]. In the setting of a tumor that is successful in suppressing the immune reaction against it, this balance would be shifted toward a T regulatory-dominated response. As long as the T effector response is initiated, the $CD8^+$ T cells will find their way to the area of interest, infiltrate the tumor and, through the T-cell receptor (TCR), bind to the appropriate antigen on the cancer cells and initiate the elimination of these cells[147,149].

If cancer cells are to form and progress, it is imperative to manipulate and evade the immune response described above and eventually establish an immunosuppressive tumor microenvironment (TME). This is a complex process in which chemokines and other cytokines are key factors in creating pro-tumor conditions.

Chemokines

Chemokines are small chemotactic protein ligands that are secreted from a wide array of immune cells and serve to recruit immune cells from the bone marrow under both homeostasis and states of inflammation[150]. Like many other immune mediators, these ligands are capable of exerting both anti-tumor and pro-tumor influences. Chemokines are divided into four groups depending on the position of a cysteine residue in the protein (XC, CC, CXC, CX3C) and act by binding specific cell surface receptors on the target cell and are also heavily involved in interaction with the extracellular matrix[151]. Chemokines primarily associated with Th1 immune responses such as CXCL9 and CXCL10 attract CD8+ T cells and natural killer cells (NK) to tumor areas where their presence is associated with retarded tumor progression[152,153]. On the other hand, chemokines can promote tumor progression through the recruitment of T regulatory cells (T reg) that have an affinity for CCL22, which tumor cells secrete[154]. This can lead to the suppression of the immune response to the tumor and promote tumor progression and metastasis[155,156]. Other cells such as tumor-associated macrophages (TAM) and DCs are drawn to the TME by chemokines exerting a pro-tumor effect[154,157]. In addition, the chemokines can directly influence tumor cells and the surrounding stroma and promote tumor proliferation and angiogenesis[158].

In recent years, the key function of chemokines in tumor progression has drawn attention to their role as both biomarkers and targets for therapeutic intervention. CXCL5, for example, is important for crosstalk between tumor cells and host stroma and has been investigated as a predictor of survival in a number of studies on different types of cancer. These studies have collectively shown that increased expression levels of CXCL5 either in tissue samples or plasma are associated with poor survival. This chemokine could therefore function as a valuable biomarker of prognosis in addition to its potential as a therapeutic target[159,160].

Interleukins

Like chemokines, interleukins play an integral part of cell communication in the TME, acting as both pro-inflammatory and anti-inflammatory interleukins. Certain interleukins such as IL-1β and IL-6 are known to promote cancer growth, whereas other interleukins such as IL-2, IL-12 and IFN-γ have anti-tumor effects. Based on this, a number of clinical trials have previously attempted to utilize anti-tumor interleukins as therapeutic agents but with limited effect, at least as a form of monotherapy, where a significant obstacle has been achieving sufficient concentrations of these agents in the TME, as well as overcoming toxicities[161]. However, studies are ongoing on combination treatments with other immunomodulatory agents such as checkpoint inhibitors in which interleukins and other cytokines might be the key to overcoming the therapeutic resistance still seen in many cases[161,162]. Targeting immunosuppressive pro-tumor interleukins has also been studied, including IL-6 receptor blockade, which is an established treatment option for rheumatoid arthritis[163].

Growth factors

After their formation, tumors largely rely on growth factors for their continued development. A number of different growth factor groups are known to be a part of tumor progression. The epidermal growth factor (EGF) family has been relevant in the context of HNC as cetuximab, an EGFR inhibitor previously discussed, is indicated for patients that are not expected to tolerate cisplatin. Both the vascular endothelial growth factor (VEGF) and the fibroblast growth factor (FGF) stimulate abnormal neoangiogenesis, which is essential for tumor growth[164]. The interception of growth factors or their respective receptors has shown promising results and FGF receptor (FGFR) blockade is now approved for both urological malignancies and cholangiocarcinoma[165]. Studies have shown a correlation between the overexpression of FGFR and poor outcome in HNC and, on the basis of promising pre-clinical results, a number of clinical trials on FGFR inhibitors are ongoing[166].

Immune response to treatment

There is ample evidence to show that cancer treatment affects the immune system[167,168]. This applies to both pharmaceutical therapy and RT, and even surgery, to a lesser extent. The immune reaction that is initiated by the treatment is often both beneficial and necessary for achieving tumor breakdown, although it can be a two-edged sword as the inflammation that follows contributes to the disease-related systemic inflammation that is already present with circulating cytokines and chemokines. This combination can be harmful and contribute to side effects such as cachexia and trismus, and might even negatively impact the prognosis[169,170].

The immunomodulatory effects of RT on tumor cells and the TME have been well documented. Ionizing radiation can induce immunogenic cell death in various ways including the release of antigens from tumor cells after they die, which can then be processed by antigen-presenting cells. Furthermore, the tumor cell, although surviving the radiation, can be subject to changes that make it visible and susceptible to immune-mediated killing[167,171]. The immunogenic properties of RT and cisplatin as well make them potentially attractive partners for immunotherapeutic agents[172-174]. Checkpoint inhibitors and other immunotherapeutic agents depend on the significant presence of immune cells in the TME in order to achieve their goal. Immunologically cold tumors, i.e., where the TME is sparsely populated by immune cells and is dominated by stroma, are usually resistant to immunotherapy, but a combination of chemotherapy and/or radiotherapy has the potential to open up these tumors up to an influx of immune cells such as cytotoxic CD8+ T cells[175,176].

Further evidence of the systemic inflammatory reaction induced by RT is the so-called abscopal effect, where evidence of the regression of metastatic disease can be seen outside the field of irradiation[177]. This effect is certainly immune mediated and can best be described as an in-situ cancer vaccination created by the RT[178]. Previously considered quite rare and sporadic, the breakthroughs in immunotherapy in recent years have put the abscopal effect in a different light, although the results of pre-clinical experiments have yet to be translated into clinical trials[179].

Proteomics and biomarkers

The term *proteomics* applies to the large-scale analysis of the proteome, which refers to all proteins expressed by the genome and can be seen as an extension of genomics. Compared to genomics, however, proteomics is even more complicated due to the vast number of proteins in the human body and the variety of post-translational modifications that take place. Proteomics can be divided into expression proteomics, which encompasses qualitative and quantitative expression analyses, structural proteomics, which focuses on the three-dimensional structure and variations in proteins, and finally, functional proteomics, in which the functions and interactions of proteins are analysed[180,181].

The advent of proteomics at the turn of the century has opened up the field of protein biomarker discovery and liquid biopsies, especially with the recent advancements of high multiplex technology, which is capable of analyzing large sets of proteins in small biological samples. The most commonly used technologies are mass spectrometry and affinity proteomics, which utilizes antibodies for protein detection[182].

Biomarker detection using proteomics can be divided into discovery proteomics and target proteomics, in which the former is non-hypothesis driven and the latter has a validating and hypothesis-driven goal [183]. Biomarkers for detection of disease, prognosis and treatment response are scarce in HNC and EBV-DNA for detection of recurrent EBV positive nasopharyngeal cancer is currently the only biomarker in clinical use[184]. Extensive research has been conducted on HPV-DNA for liquid biopsy in all HPV-associated types of cancer, including OPC but thus far has not been implemented in clinical practice[185,186]. The literature contains many studies that have identified various plasma protein biomarkers associated with HNC, but no suitable candidates are currently in sight[187]. Salivary biomarkers have also gained interest for the possible detection of OCC and OPC, especially inflammatory cytokines, but the increased expression of these cytokines in benign inflammatory diseases of the mouth might limit the use of this method for the early detection of cancer in the future[188-190].

Body mass composition

In its simplest form, body mass composition (BMC) consists of fat and fat-free mass (FFM) that includes skeletal muscle mass (SMM). FFM can also be represented as a fat-free mass index (FFMI; FFM/m^2) and is affected by various conditions, including cancer. A high proportion of HNC patients have lost a considerable amount of muscle mass and therefore also FFM at diagnosis[191]. This is due to, for example, reduced energy intake because of dysphagia and a systemic inflammatory condition caused by the cancer. The majority of patients with HNC lose body weight during RT and this body weight loss is largely based on the loss of SMM[192,193].

In many ways, malnutrition is the other side of the same coin as body mass composition and has been defined and assessed in a clinical context in different ways. The Global Leadership Initiative on Malnutrition (GLIM) was implemented to form a global framework of malnutrition definitions and guidelines[194]. According to the GLIM, the individual in question should be screened for malnutrition using validated tools and be subject to further assessment if deemed at risk of malnutrition. It is proposed that the diagnosis of malnutrition is constructed from three phenotypic criteria (weight loss, low body mass index (BMI), reduced muscle mass) and two etiologic criteria (reduced food intake, disease burden/inflammation)[194]. To acquire a diagnosis of malnutrition there must be at least one phenotypic and one etiologic criterion. The severity of malnutrition is then graded according to the phenotypic criteria and this can be conducted using different scales. The cut-off values for phenotypic criteria vary across screening tools but a common cut-off value for weight loss is >5% weight loss in 6 months for BMI <20 kg/m^2 and, for FFMI, a cut-off value of <17 FFM/m^2 for males and <15 FFM/m^2 for females is usual[195].

There are several methods for measuring BMC. The gold standard is considered to be dual-energy X-ray absorptiometry (DEXA) but this method exposes the individual measured to radiation. A commonly used method, at least in the literature, is the measurement of muscle mass at the level of the third lumbar vertebrae on CT, which requires abdominal series in the exam[196]. Bioelectrical impedance analysis (BIA) is widely used for measuring BMC in different settings. With this method, the resistance (dependent upon intracellular and extracellular fluid) and reactance (dependent upon the cell membrane) of body tissue are measured by passing an electrical current between two electrodes, most commonly placed on the hand and foot[197]. Values for FFM and FFMI can be derived from the results. The use of BIA is non-invasive with no risk for the patient and is easy to apply as long as the patient is not bedridden. BIA, however, is contraindicated in patients with pacemakers and implantable cardioverter defibrillators. The method has some limitations when compared to the gold standard as it is an indirect measurement of BMC. It has been reported that variations in the state of hydration of the individual might affect the results and that BIA systematically underestimates FFM[198]. Moreover, its utility in critically ill patients is still uncertain[199]. It can, however, be argued that a lack of standardization of the BIA protocols used in different settings hinders comparison between studies and minor statistically significant differences in the values calculated do not necessarily become clinically relevant[200]. This problem could be eliminated by implementing mandatory international standards for manufacturers of BIA devices[198]. An example of a BIA device is shown in Figure 3.

Figure 3 – BC-418MA, Tanita Corporation, Tokyo, Japan.

Research aims

General aims

The main theme of this doctoral thesis is local and systemic inflammation related to HNC and its treatment. The four studies investigate different manifestations and markers of inflammation with the general aim of gathering more information about the effects of inflammation on survival and exploring the dynamics of protein biomarkers related to treatment. An overview of Papers I-IV is shown in Table 1.

Table 1 - Overview of study designs for Papers I–IV.

Paper I	**Prospective observational study**	**244 patients** **127 patients completed study period**	**Mouth opening measured before radiotherapy and 2, 6, and 12 months after completion of radiotherapy**
Paper II	**Prospective exploratory study**	**30 patients**	**Measurement of 10 cytokines and 4 growth factors before treatment, 7 weeks after the start of treatment and 3 and 12 months after the termination of treatment**
Paper III	**Prospective observational study**	**180 patients**	**Proteomics measurement of 83 cytokines before treatment, 7 weeks after the start of treatment and 3 and 12 months after the termination of treatment**
Paper IV	**Prospective observational study**	**404 patients**	**Body mass composition determined with bioelectric impedance analysis before treatment**

Specific aims

Paper I

To assess the incidence of trismus and its impact on 5-year overall survival rate in a cohort of patients receiving radiotherapy, either as single modality treatment or in combined modality treatment, for head and neck cancer.

Paper II

To assess the longitudinal expression levels of inflammatory cytokines and growth factors in serum before treatment and up to one year after treatment for head and neck cancer.

Paper III

To evaluate the expression of inflammatory proteins in serum before and up to one year after treatment and to assess whether there were any differences in protein expression between treatment modalities.

Paper IV

To investigate how pretreatment body mass index and fat-free mass index correlate with early death in patients with head and neck squamous cell carcinoma and a WHO performance status of 0–2, and whether these measurements can be used as markers of prognosis for risk stratification.

Materials and methods

Paper I

This study included 244 patients with HNC who received 3DCRT. RT was given either pre-operatively (n=84), post-operatively (n=86) or as a primary treatment (n=74). An early rehabilitation program was used in which all patients were seen by a physiotherapist and instructed in oral mobility and jaw-opening exercises with the Acute Medic Jaw Trainer and Stretcher®. The patients met with the physiotherapist on four occasions – before RT and 2, 6, and 12 months after the termination of RT. The maximum interincisal opening (MIO) was measured before RT and at all three follow-ups. Trismus was defined as MIO ≤35 millimeters and overall survival was computed with the Kaplan-Meier estimate. The study was approved by the Regional Ethical Review Board in Stockholm and no ethical approval was required as the study was identified as a clinical development program (2005/767-31/1-4).

Paper II

This exploratory study was part of a Swedish multicenter study in which patients were prospectively recruited at three tertiary hospitals. Thirty patients about to undergo curative treatment for HNC were included in this cohort. The most common subsites were oropharynx (n=13) and oral cavity (n=9). Blood was drawn on four occasions: before the initiation of treatment, 7 weeks after the start of treatment, 3 months after the termination of treatment and finally 12 months after the termination of treatment. Serum was analyzed for cytokines interleukin-1β (Il-1β), interleukin-2 (Il-2), interleukin-4 (Il-4), interleukin-5 (Il-5), interleukin-6 (Il-6), interleukin-8 (Il-8), interleukin-10 (Il-10), granulocyte-macrophage-colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), and interferon-γ using the 10-plex ultra-sensitive cytokine assay (Thermo Fisher Scientific, Waltham, MA, US) and growth factors granulocyte colony stimulating factor (G-CSF), fibroblast growth factor 2 (FGF-2), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) using Luminex xMAP technology (Merck Millipore, Darmstadt, Germany). The Regional Ethical Review Board in Uppsala reviewed and approved the study (no. 2014/447).

Paper III

This study was part of the same multicenter study as in Paper II. A total of 180 patients with curable HNC were included in this cohort, including the 30 patients from Paper II. The most common sites were oropharynx (n=81), oral cavity (n=53) and larynx (n=22). The cohort was divided into four groups depending on treatment modality: surgery only (n=24), RT+/- surgery (n=94), RT and cisplatin chemotherapy +/- surgery (n=47), RT and EGFR monoclonal antibody +/- surgery (n=15). Blood was drawn on four occasions: before treatment, 7 weeks after the start of treatment, 3 months after the termination of treatment, and 12 months after the termination of treatment. Thus, Paper III shares the same basic structure as Paper II but in Paper III the expression of 83 proteins known to be immuno-oncologically significant was measured using a different technique, an affinity-based proteomics biomarker panel (Olink, Uppsala, Sweden) in a much larger cohort. This high-throughput technology called *Proximity Extension Assay* (PEA) requires dual binding of DNA-tagged antibodies to proteins, thereby assuring specificity. The DNA sequence formed through hybridization is unique to each protein. PCR amplification is performed and the number of PCR cycles is directly related to the expression levels of the proteins. These data are then processed resulting in the relative concentrations of each protein in the sample, presented as normalized protein expression (NPX), an arbitrary unit on the Log2 scale[201]. The PEA process is illustrated in Figure 4. The Uppsala Regional Ethics Review Board reviewed and approved the study (no. 2014/447).

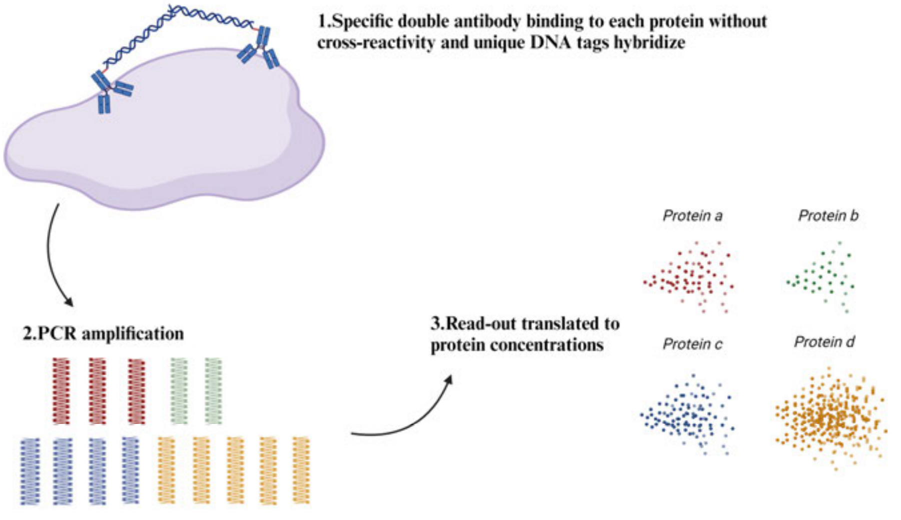

Figure 4 – Schematic illustration of Proximity Extension Assay. Modified from www.olink.com and created with BioRender.com.

Paper IV

Like Papers II and III, this paper was conducted within the same Swedish multicenter study. After exclusion, the cohort consisted of 404 patients with curable HNSCC. The most common sites were oropharynx (n=185), oral cavity (n=113), and larynx (n=47). All 404 patients underwent BIA at diagnosis and were followed up for at least 12 months. BIA was measured with an 8-electrode device (BC-418MA, Tanita Corporation, Tokyo, Japan). FFMI was calculated as FFM divided by the square of height (FFM/m2). Body weight loss during the 6 months leading up to diagnosis was reported by the patients. BMI was calculated during all visits. To diagnose malnutrition according to GLIM prior to the start of treatment, phenotypic and etiologic criteria were used[194]. Phenotypic criteria were self-reported body weight loss (either >5% within the past 6 months or >10% beyond 6 months), BMI (<20 kg/m^2 if <70 years or <22 kg/m^2 if >70 years) or FFMI measured by BIA (<17 FFM/m^{2}for males and <15 FFM/m^2 for females)[202,203]. The etiologic criteria were partial or no food intake with a need for nutritional support or C-reactive protein (CRP) >5 mg/L. The Uppsala Regional Ethics Review Board reviewed and approved the study (no. 2014/447).

Results

Paper I

A total of 244 patients were recruited for this prospective study and 127 of them completed the study period of 12 months. Of the 117 patients who dropped out of the study, 51 did so because of tumor recurrence, or were deceased. Information regarding the drop-out for 9 patients was missing and the remaining 57 patients declined further participation at different stages of the study. For the whole group, a decline in mouth opening (MIO) was noted initially. Gradual recovery was seen at the final control at 12 months. At baseline, 34 patients (13.9%) had trismus with an MIO equal to or less than 35 mm. The mean MIO at baseline was 46 mm (8–74 mm) and reached the lowest point at 2 months after completion of treatment, 39 mm (14–69 mm). At 12 months after completion of treatment the mean MIO was slightly higher, 40 mm (5–67 mm), and 30% of the patients measured at this time point had trismus, which was most prevalent in patients with OCC (44%) followed by patients with OPC (37%). At 12 months, 80% of the patients had decreased MIO compared to baseline before treatment. Patients with trismus at 12 months after the termination of treatment had a tendency toward a worse overall 5-year survival rate than patients without trismus, but this was not statistically significant (p=0.64)

Paper II

In general, for the cohort, an increased expression of IL-1β, IL-6, and IL-10 was seen at 7 weeks after the start of treatment compared to baseline. These cytokines had returned to pre-treatment levels at the next measurement at 3 months after treatment had ended. IL-1β and IL-10 slightly increased again in the final measurement at 12 months after treatment. When the cohort was stratified into three groups depending on treatment modality (surgery only, RT with or without surgery, CRT with or without surgery), patients who had received CRT had higher expression levels of IL-1β, IL-6, and IL-10 than the other groups at 7 weeks after the start of treatment. Nine out of 10 patients in the CRT group received cisplatin and one patient received cetuximab but was grouped under chemotherapy in this study.

Four patients experienced recurrence within 12 months of termination of treatment. These patients had higher expression levels of IL-1β, IL-6, IL-8, and IL-10 than the remaining patients at 7 weeks after the start of treatment. This difference was not seen in the other three measurement time points. Patients with a more advanced stage (T3-T4) had higher expression levels of IL-1β and IL-8 at the 7-week time point.

With regards to growth factors, fewer variations were seen than with cytokines. However, the levels of G-CSM were shown to be higher at 7 weeks in the patients who developed recurrence.

Paper III

For the entire cohort, variations in protein expression were most pronounced around the time of treatment. The expression levels of 15 proteins changed significantly at 7 weeks after the start of treatment compared with the baseline pre-treatment values. CCL20, Fas ligand (FASL) and MMP20 exhibited the most significant decrease in expression at 7 weeks, while IL-6 and IL-10 exhibited the most significant increase in expression (Figure 5). CCL20 and ARG1 remained significantly decreased and CCL17 increased compared to baseline at all time points.

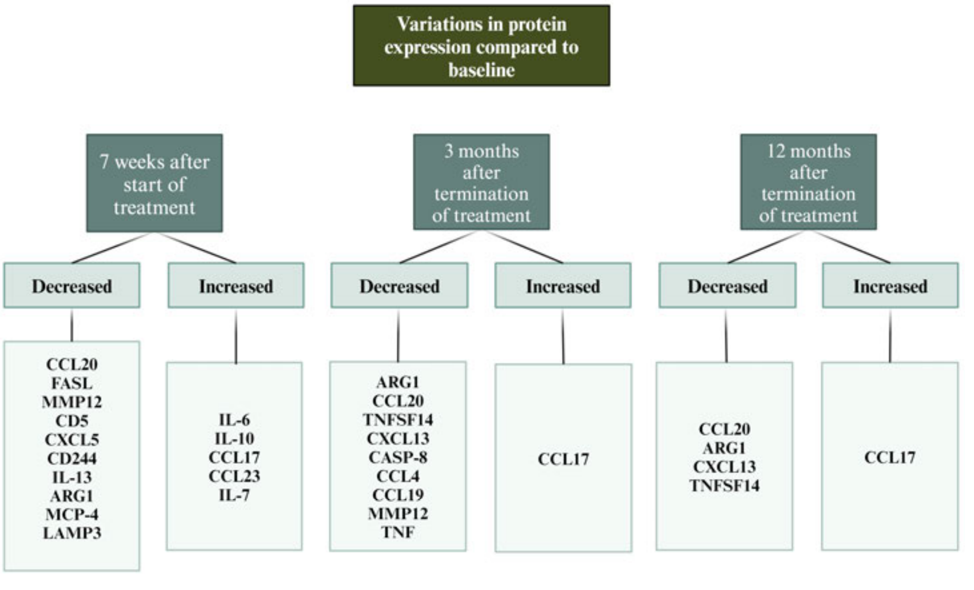

Figure 5 – Variations in protein expression at three time points compared to baseline before treatment in decreasing order of significance. Created with BioRender.com.

A significant difference in the expression of 17 proteins was observed between the four treatment groups. A multivariate analysis demonstrated that three of these proteins had a stronger correlation with mucositis (IL-6) and smoking pack years (IL-10 and IL-18) than the treatment modality. Hence, as a result of the multivariate analysis, the expression of 14 proteins correlated most strongly with the treatment modality of which 13 proteins had significantly lower expression in the CRT group compared with the RT group at 7 weeks after the start of treatment (Figure 6).

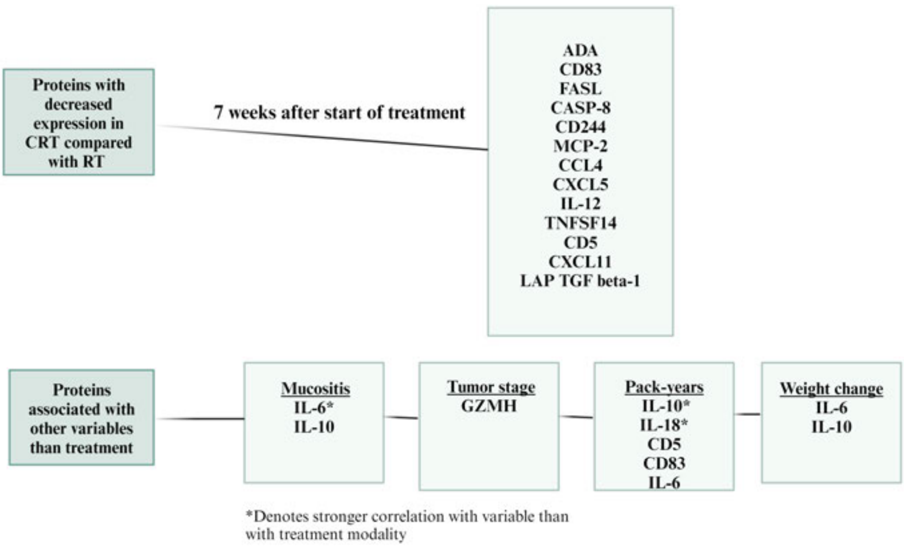

Figure 6 – Association of protein expression with chemoradiotherapy and other variables according to a multivariate analysis. Created with BioRender.com.

Paper IV

According to self-reported weight loss within 6 months prior to diagnosis, the mean body weight loss for the cohort of 404 patients was 1.9 kg. Patients with more advanced stage disease (stage III and IV) reported significantly more weight loss prior to diagnosis than patients with less advanced stage disease (stage I and II), 2.7 kg and 1.4 kg, respectively (p=0.006). For the entire cohort, 79 patients (19.6%) had non-response to treatment (residual disease or recurrence) within one year of its initiation. For this group, both weight loss and loss of BMI prior to diagnosis were significantly higher than for the responders with loco-regional control one year after the start of treatment (2.9

kg and 1.7 kg, p=0.005; 1.0 kg/m2 and 0.56 kg/m2, p=0.004). Non-response was more frequent for patients with malnutrition at diagnosis according to the GLIM criteria, compared with patients without malnutrition at diagnosis (31% and 17%, respectively, p=0.002).

Ten patients (2.2%) died within 6 months of the start of treatment and within one year 25 patients had died (6.2%). Patients who died within 6 months of the start of treatment had significantly lower FFMI at diagnosis compared with patients who survived beyond 6 months (17.6 kg/m2 and 19.5 kg/m2, respectively, p=0.035). BMI at diagnosis, however, did not differ between these two groups. When patients who died within 12 months were compared with patients who survived beyond one year, FFMI at diagnosis was again significantly lower for non-survivors (p=0.005) but there was no difference in BMI at diagnosis between the groups. Malnutrition at diagnosis correlated significantly with death within 12 months (p<0.001) but was not shown to be an independent prognostic factor.

Discussion

This thesis is based on different manifestations and aspects of systemic and local inflammation in HNC. The first paper tackles the consequences of radiation-induced inflammation in the masticatory apparatus and its effects on survival, while the second and third papers explore the systemic immune response that is caused by cancer and its treatment. Finally, the main focus of the fourth paper is abnormalities in body mass composition at the time of diagnosis, especially the relation to early death in HNC.

Paper I

This study demonstrated that patients with OCC and OPC are at greatest risk of radiotherapy-induced trismus and should be considered for jaw-opening exercises during and after treatment. Trismus at 12 months was especially pronounced for OCC patients as 44% were found to have MIO at or below 35 mm. These findings are similar to previous research in this field[126,128]. As previously described, the incidence of trismus in HNC has been difficult to pinpoint due to the heterogeneity of study designs. Close to one third of the patients who completed the study presented in Paper I had trismus at 12 months after the completion of treatment, which must be considered a high number, especially given that the entire cohort was instructed in jaw-opening exercises. However, it should be noted that compliance was not controlled.

Regarding the prevention of trismus, compliance is indeed a problem and patients for these exercises should be carefully chosen and include those patients who stand to benefit the most. A significant finding was that patients with a greater baseline mouth opening before treatment were shown to have the greatest reduction in mouth opening after treatment and, in a way, these patients stand to lose the most, even if they do not end up with trismus, according to the formal definition. Thus, offering mouth opening exercises during treatment to patients with MIO values that are far from the trismus cut-off could be of value.

An obvious connection between trismus and survival might not be apparent and the literature does not contain any other studies highlighting this connection. However, trismus can be a debilitating handicap that sometimes leads to malnutrition and increases the risk of aspiration. In addition, decreased mouth opening often prevents the sufficient visualization of areas of oncological interest in the oral cavity and oropharynx, which could lead to delayed diagnosis of tumor recurrence. In our study we observed a tendency toward a worse five-year overall survival rate in patients with MIO at or below 35 mm after 12 months of treatment, but the difference was not significant. Thus, further studies are required to establish trismus as a predictor of survival.

All patients in this study were treated with 3DCRT which, in most centers, has been replaced by IMRT and VMAT. These advances in radiotherapy produce, at least theoretically, a milder negative effect on the masticatory apparatus and should result in a lower incidence of trismus. De-escalation in the treatment of HPV positive OPC might decrease the incidence of trismus, however, at the present time a consensus has not been reached regarding this topic[204].

One of the strengths of this study is the relatively large cohort that was followed for 12 months, despite a drop-out rate of 48%. It could also be postulated that the remaining patients who completed the entire study period were much more likely to adhere to the mouth opening exercise regimen than their counterparts who dropped out. A randomized control study with a more uniform cohort may have resulted in a deeper understanding of the effectiveness of mouth opening exercises.

Papers II and III

The main purpose of Papers II and III was to explore how cytokines and growth factors (Paper II) and a series of inflammatory proteins (Paper III) evolve over time in relation to the treatment of HNC patients. Studies evaluating peripheral cytokine profiles in HNC are scarce and there needs to be a deeper understanding of the immune reaction that takes place in HNC patients undergoing treatment. There is also a scarcity of biomarkers in HNC for both detection of disease and prognosis.

Paper II

Paper II investigated a field that is sparsely documented and can therefore be described as an exploratory study. The cohort consisted of 30 unselected patients with tumors in different locations about to undergo treatment with curable intent. The elevated expression levels of IL-1β, IL-6 and IL-10 for the entire cohort at 7 weeks, at the end of radiotherapy and chemoradiotherapy for the majority of the patients, were indicative of a clear effect of these treatment modalities on the immune system. The chemoradiotherapy group in particular exhibited a significantly greater expression of these cytokines at this time point compared with patients who were only surgically treated or received radiotherapy without chemotherapy. Nine of the 10 chemoradiotherapy patients were treated with cisplatin and this finding adds further evidence for the immunomodulatory effect of this agent.

When cytokine expression levels were correlated with recurrence within one year after termination of treatment, the same above-mentioned cytokines and IL-8 were found to be significantly higher at 7 weeks for the patients who developed recurrence. IL-1β and IL-8 also correlated with T-stage, which could indicate a more powerful immune response to the breakdown of larger tumors. These four cytokines might therefore be of interest to further research as markers of prognosis, as well as G-CSM, which was found to be higher in the recurrence group at the same time point. The most likely confounder to influence cytokine expression in patients undergoing radiotherapy and chemoradiotherapy is mucositis. However, in this study, no correlation was found between the two at 7 weeks.

IL-6 is a pro-inflammatory cytokine that has been linked to poor prognosis in various types of cancer, including HNC, where it has been proposed as a potential prognostic marker, while IL-8 has previously been implicated in the prognosis of both nasopharyngeal cancer and esophageal cancer [205-208]. The role of IL-1β in the pathogenesis of OCC has been studied and this cytokine, which is highly pro-inflammatory, seems to be central in this process. Its potential as a salivary biomarker has been discussed but whether it carries the same potential as a peripheral blood biomarker remains to be seen[209-211]. The assessment of elevated IL-10 in this context is not straightforward as it is considered to have a dual role in cancer, acting as both an anti-tumor and a pro-tumor cytokine depending on the surrounding cytokine milieu[212].

Paper III

This study can be seen as an expanded version of the study in Paper II. Using a different and more potent method for protein analysis, the expression levels of 83 immuno-oncologically significant proteins were determined at four time points for 180 HNC patients. Again, significant dynamics in protein expression were seen, primarily close to the end of radiotherapy and chemoradiotherapy. Fifteen proteins had either decreased or increased expression levels at 7 weeks compared to pre-treatment expression levels. At 3 months after the termination of treatment, 11 of these proteins had returned to similar expression levels as pre-treatment expression levels, reflecting a regression of a systemic inflammation in the cohort, both cancer and treatment related.

Indeed, treatment with radiotherapy with concomitant cisplatin in particular was shown to be connected to significantly decreased expression of 13 proteins at 7 weeks after the start of treatment compared to radiotherapy. Cisplatin has been the cornerstone of chemotherapy in HNC for many decades and it is becoming increasingly clear that it not only acts through cytotoxic DNA and mitochondrial damage, but also through modulating the immune response, not only locally in the TME but probably systematically as well. In vitro studies have shown that cisplatin results in the up-regulation of major histocompatibility complex I (MHC class I) in tumor cells, which is of great importance for antigen presentation to cytotoxic T cells[168,213,214]. Among the other effects of cisplatin are attracting effector cells to the TME and enhancing the cytotoxic effects of T cells at the molecular level, as has been demonstrated in a number of pre-clinical studies[215,216]. It could be concluded that the optimal net effects of cisplatin are not only cancer cell death but also the abolishment of the pro-tumor immunosuppressive conditions in the TME, which encourages combination treatment with immunotherapy.

Molecular changes in the TME are not necessarily reflected peripherally but it is probable that the expression of proteins in the blood compartment to a certain degree indicates the local chain of events around the cancer cells. Moreover, even though the blood immune response is not a clear reflection of the TME, it is becoming increasingly apparent that what happens peripherally in the blood impacts the mechanisms of the anti-tumor effects exerted by the immune system and immunotherapeutic agents. The effects of cisplatin and radiotherapy involve the liberation of so-called damage-associated molecular patterns (DAMPs) from cancer cells in stress, which triggers the innate immune system and also the adaptive immune system through cytokine release, evident in the blood compartment. This reaction can be detrimental and contribute to radiation injury. However, in the context of HNC therapy, a deeper understanding of these processes is required[217].

The 13 proteins that had decreased expression in the blood compartment of patients receiving chemoradiotherapy have various functions in an immuno-oncological context. IL-12, for example, is primarily secreted by DCs and considered pro-inflammatory in nature with potent anti-tumor effects[218,219]. What lies behind its decreased expression in the CRT group could be subject to speculation but increased consumption in the TME might be one explanation. FASL plays an important role in cell apoptosis including that of peripheral T cells by activating the transmembrane protein FAS. The literature has reported conflicting results on the significance of serum FASL levels in HNC and one study of 37 HNC patients found that lower levels of FASL indicated active disease, while another study of OCC patients failed to establish a similar connection[220,221]. Likewise, although considered an attractive therapeutic option in theory, in order to induce cancer cell death, the future role of FASL in the treatment of cancer is thus far uncertain, given that there is in vitro evidence that FAS/FASL activation could have a paradoxical effect on tumors and promote their growth instead of their reduction[222]. FASL has been proposed as a biomarker for both thyroid cancer and HNC and has shown promising results but further research is warranted[223,224]. As previously described, CXCL5 is a chemokine of interest with potential as a biomarker and was significantly lower in the cisplatin-treated group at 7 weeks. As high levels of this chemokine have been associated with poor prognosis, this finding could reflect a positive therapeutic immunomodulatory effect of cisplatin.

Paper IV

In this study, which is one of the largest prospective studies on body mass composition in HNC patients to be conducted thus far, 404 patients with HNSCC were evaluated with BIA before treatment. FFMI at diagnosis predicted early death within 6 months and also within 12 months. A number of previous studies have been conducted on this subject although other methods of BMC evaluation were used in most of the studies. In a retrospective cohort of 190 patients, Grossberg et al. found that low skeletal muscle mass based on CT before treatment was a predictor of overall survival, although early death was not specifically studied as an endpoint[225]. In addition, a higher pre-treatment BMI was found to be a predictor of improved survival, although this finding was not reproduced in our study. Another recent retrospective study on 71 HNSCC patients found a significant correlation between low FFMI measured with BIA and overall survival[226]. Thus, the findings presented in Paper IV further strengthen the importance of FFMI as a prognostic factor, especially for early death, which is an important parameter to include in the prognosis before treatment. A negative body mass composition at presentation is likely indicative of an underlying cancer-related systemic inflammation as well as dysphagia, often for a prolonged period leading up to the diagnosis. A logical explanation for the relationship between low FFMI and early death can probably be found in the systemic immune response elicited by the cancer. An aggressive tumor with worse short-term and long-term prognosis might induce a more powerful host immune response and subsequently lead to a higher degree of muscle wasting and low FFMI before treatment is initiated.

Malnutrition correlated with death within 12 months but was not an independent prognostic factor according to a multivariate analysis. In the study cohort, 11.4% of the patients were already malnourished at diagnosis, which is comparable, albeit with a slightly lower prevalence, to other studies found in the literature. Variations in the diagnostic criteria of malnutrition used and the study cohorts may have influenced this outcome[227].

The terms *malnutrition*, *cachexia* and *sarcopenia* are sometimes used interchangeably, which is inaccurate. Malnutrition in the context of cancer is the result of decreased intake of energy, which can be caused by, for example, decreased appetite, depression and tumor-related dysphagia. Cachexia is a more advanced stage of muscle wasting in which systemic inflammation plays a greater role and nutritional therapy may not be sufficient to reverse such a process. All patients with cachexia probably also have malnutrition, although all patients with malnutrition are not necessarily cachetic[228]. Sarcopenia, meaning reduced muscle mass, is generally more common in the elderly, and such patients are not necessarily cachectic or malnourished.

The main advantages of BIA for the evaluation of body mass composition are its non-invasiveness and low cost. BIA devices are widely available and do not involve any risk for the individual, with the exception of patients with pacemakers and implantable cardioverter defibrillators where the use of BIA is contraindicated. To effectively assess SMM with CT, abdominal series are necessary and are rarely routinely used in HNC and, like DEXA, exposes the patient to radiation. In light of the findings of the study, as well as previous findings, it could be recommended that BIA is incorporated into the initial assessment of patients with newly diagnosed HNC, especially patients deemed at risk of malnutrition and poorly prepared for a demanding treatment and the systemic inflammation that follows. If decreased FFMI is detected, the patient should be considered a candidate for more intense nutritional and physical therapy before treatment, for example, prehabilitation and perhaps more frequent follow-ups after treatment due to the increased risk of early death.

General discussion and future perspectives

As a clinician stepping into the academic world during my doctoral studies, an important aspect has been to develop a deeper understanding of the scientific approach to study clinical issues. The overarching goal of this doctoral project was to improve knowledge of local and systemic inflammation related to HNC and its treatment. The papers in this thesis are all based on prospective observational studies and in three out of four of the papers on relatively large clinical cohorts. It is often argued that even a large longitudinal observational study with repeated measurements has significant limitations, for example, selection bias and limitations in investigating causality. However, it is also possible to adopt a more positive view of the applied design and describe how the results of this thesis have been generated by real-world studies on unselected and somewhat heterogeneous patient cohorts that were followed outside the confines of rigid controlled studies. The data generated and analyzed from our cohorts present several new findings that provide valuable information and can generate hypotheses for future clinical studies.

The results from Paper I underline the importance of rehabilitation and even prehabilitation in the prevention of trismus. It could also be concluded that the selection of patients for jaw-opening exercises is a key factor as these exercises can be time consuming, while adherence to preventive activities remains a challenge. It seems that patients with OCC and OPC could benefit most from a jaw-exercise program, although the timing and intensity of such a program need to be better studied. Even though the study could not unequivocally confirm that trismus affects the 5-year survival rate, an interesting tendency toward that was seen. However, an even larger study is warranted to investigate this relationship further.

The hypothesis-generating research presented in Papers II and III draws attention to a number of proteins that could be of interest for further investigation as potential biomarkers for treatment response and prognosis. These studies share a novel approach in the field of head and neck cancer research. Paper III, in particular, with its substantial cohort, has the potential to contribute to deeper knowledge of the immunological mechanisms at play. It is apparent that the path from results, such as those presented in Papers II and III, to a definitive confirmatory study is long and tortuous. However, subsequent studies in which the protein milieu in the TME is profiled in tumor biopsies and compared with the peripheral cytokine response would be of great value, and a necessary stepping stone. The insights gained from such a study could demonstrate if and how the immunological interplay around cancer cells manifests in the blood compartment. Designing such a study with more homogenous cohorts would be beneficial and a comparison between the peripheral immune response and healthy controls would likely further highlight certain proteins.

With regard to the value of FFMI at diagnosis shown in Paper IV and its prognostic superiority over BMI, the measurement of body mass composition with BIA could be considered a routine, cost-effective procedure in the work-up of newly diagnosed HNC patients. Further investigation of the relationship between serial BIA measurements during and after treatment and long-term prognosis is the logical next step. The connection between systemic inflammation measured in the blood compartment and changes in body mass composition is highly intriguing and could be the subject of a future study.

Conclusions

Paper I

- Patients with trismus show a tendency toward a worse overall 5-year survival rate
- Jaw opening exercises should primarily be offered to patients with OCC and OPC

Papers II and III

- Expression of inflammatory proteins in the blood is most pronounced at the end of treatment
- Cisplatin has a significant immunomodulatory effect
- Several proteins exhibited treatment-related dynamics and might be of interest for further research as potential biomarkers

Paper IV

- Low FFMI measured with BIA is an independent prognostic factor for early death within 6 and 12 months
- Decrease in body weight and BMI 6 months prior to diagnosis and malnutrition at diagnosis is a predictor of a worse response to treatment

Svensk sammanfattning

Huvud- och halscancer är ett samlingsbegrepp för maligna tumörer i läpp, munhåla, svalg, näsa och bihålor, spottkörtlar och struphuvud. Incidensen har ökat och numera diagnostiseras omkring 1 700 människor i Sverige med huvud- och halscancer varje år. Behandlingen består som regel av kirurgi och/eller radioterapi med tillägg av cytostatika vid mer avancerat stadium. Många av patienterna befinner sig i ett tillstånd av systemisk inflammation med malnutrition vid diagnos, och själva behandlingen, radio- och kemoterapi, ökar ofta denna inflammation vilket bidrar till ytterligare malnutrition och viktnedgång. Radioterapi kan även leda till lokal inflammation som kan vara gynnsam och motverka tillväxt av cancerceller, men kan även leda till biverkningar såsom minskad gapförmåga (trismus). Detta avhandlingsarbete handlar i grund och botten om systemisk och lokal inflammation hos patienter med huvud- och halscancer och dess konsekvenser, vilket är ett relativt outforskat område där det föreligger stort behov av djupare kunskap.

I första delarbetet ligger fokus på trismus och dess påverkan på 5-årsöverlevnad hos patienter som genomgått behandling för huvud- och halscancer. Det är känt att radioterapi leder till lokal inflammation i tuggsystemet samt skada på små kärl och nerver. En fibrotiserande process inleds som vanligtvis kulminerar inom 12 månader efter att behandlingen är avslutad. Slutresultatet kan i många fall bli bestående trismus som definieras som ett avstånd mellan incisiver på ≤ 35 mm. Sammanlagt 244 patienter med botbar huvud- och halscancer var initialt inkluderade i denna prospektiva studie men 117 patienter föll bort från kohorten innan 12-månadersgränsen nåddes. Gapförmågan mättes vid fyra tidpunkter (innan radioterapi samt 2, 6 och 12 månader efter avslutad radioterapi) och alla patienter fick instruktioner om gapträningsövningar av en fysioterapeut. En trend identifierades mot sämre 5-årsöverlevnad hos patienter med trismus vid 12 månader efter avslutad radioterapi jämfört med patienter med normal gapförmåga, men denna skillnad hade inte en statistisk signifikans. Trettio procent av alla patienter som mättes vid 12 månader efter radioterapi hade trismus. Högst förekomst av trismus fanns hos patienter med oral cancer följt av patienter med orofarynxcancer. Eftersom gapträning kräver relativt stora insatser från individen är det viktigt att erbjuda den till patienter där den gör störst nytta, vilket utifrån resultaten i denna studie är patienter med oral cancer och orofarynxcancer.

I delarbete 2 utforskas den systemiska inflammationen genom mätningar av 14 inflammatoriska proteiner (cytokiner och växtfaktorer) i en oselekterad kohort av 30 patienter med huvud- och halscancer. Liksom de resterande arbetena i denna avhandling var delarbete 2 en del av en svensk prospektiv observationsstudie med medverkan av flera center. Mätningarna utfördes vid fyra tidpunkter: innan behandling, 7 veckor efter påbörjad behandling, 3 månader efter avslutad behandling och slutligen 12 månader efter avslutad behandling. Jämfört med före behandling fanns ett ökat uttryck av interleukin-1β, interleukin-6 och interleukin-10 vid tidpunkten 7 veckor efter påbörjad behandling, det vill säga en tidpunkt som sammanföll med avslut av radioterapi hos majoriteten av patienterna. Uttryck av dessa cytokiner återgick till grundnivåer vid 3 månader efter avslutad behandling. Patienter behandlade med kemoradioterapi hade högre uttryck av interleukin-1β, interleukin-6 och interleukin-10 än övriga patienter vid 7 veckor efter påbörjad behandling. 9 av 10 patienter i kemoradioterapigruppen behandlades med cisplatin. De fyra patienter som fick återfall av cancer inom 12 månader efter avslutad behandling hade vid 7-veckorsmätningen ett högre uttryck av samma interleukiner samt även av interleukin-8 jämfört med sjukdomsfria patienter. Delarbetet belyser effekten av cisplatinbaserad behandling på immunresponsen samt påvisar fyra interleukiner som kan vara intressanta att utforska vidare som biomarkörer.

Delarbete 3 är starkt förknippat med delarbete 2 men antalet patienter som inkluderades var betydligt högre och uttrycket av sammanlagt 83 olika proteiner av immunonkologisk betydelse analyserades. Mätningarna utfördes vid samma fyra tidpunkter men analysmetoden i delarbete 3 baserades på en proteomikteknik som har högre kapacitet än den så kallade *ultrasensitive assay* som användes för den föregående studien. Vid 7 veckor efter påbörjad behandling hade uttrycket för 15 proteiner ändrats signifikant jämfört med före behandling. De proteiner som visade mest signifikant sänkning av uttryck var CCL20, FAS-ligand och MMP20 medan interleukin-6 och interleukin-10 stack ut bland de proteiner som hade ökat uttryck. Cisplatin visade sig återigen påverka immunresponsen eftersom 13 proteiner vid tidpunkten 7 veckor uppvisade signifikant lägre uttryck i kemoradioterapigruppen än hos patienter som enbart behandlades med radioterapi. Sammantaget understryker resultatet som redovisas i delarbete 3 att cisplatin har en markant immunomodulerande effekt som är i linje med tidigare forskning, men dessa studier har till stor del varit baserade på prekliniska material. Dessutom har ytterligare proteiner för potentiell framtida biomarkörforskning inom huvud- och halscancer lyfts fram.

Huvudsyftet med delarbete 4 är att utröna om ogynnsam kroppssammansättning vid diagnos påverkar tidig död hos patienter med huvud- och halscancer. Många patienter är undernärda när diagnosen ställs och både sväljningssvårigheter samt systemisk inflammation kan ligga bakom detta tillstånd. Genom så kallad bioelektrisk impedansteknik (BIA) mättes kroppssammansättningen på 404 patienter vid diagnos. Dessutom dokumenterades patientens självskattade viktnedgång 6 månader innan diagnos. Patienter med mer avancerat stadium av sjukdom uppskattade sin viktnedgång signifikant högre än patienter med lägre stadium (2,7 kg mot 1,4 kg). Viktnedgång innan diagnos och malnutrition enligt GLIM-kriterier vid diagnos korrelerade med sjukdomsåterfall inom 12 månader efter påbörjad behandling. De patienter som avled inom 6 månader från påbörjad behandling hade signifikant lägre fettfri massa-index (FFMI) vid diagnos jämfört med patienter som överlevde 6 månader. Detsamma gällde för de som avled inom 12 månader jämfört med patienter som överlevde 12 månader. Från detta delarbete är det således tydligt att FFMI har ett starkt prognostiskt värde vad gäller tidig död hos patienter med huvud- och halscancer och mätning av kroppssammansättning kan vara en viktig del i utredningen innan behandlingsrekommendation presenteras.

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