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Aspects on Head and neck Cancer with special reference to Salivary Gland Tumours and Single Nucleotide Polymorphism

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Abstract
Cederblad, L. 2017. Aspects on Head and neck Cancer with special reference to Salivary Gland Tumours and Single Nucleotide Polymorphism. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1390. 47 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0130-3.

A thesis on Head and neck cancer focusing on dose planning, salivary gland carcinoma and Single nucleotide polymorphism.

For dose planning PET/CT (Positron emissions tomography/computed tomography) with tracer gave more precise information in comparison dose planning with CT. More primary tumours and metastases were found with the acetate tracer than with glucose tracer. Acetate PET/CT also showed larger volume of tumours attributed to lipid metabolism.

In a retrospective study salivary gland cancer 5-year overall survival (OS) was 53 %. Salivary gland carcinoma consists of many histopathological groups, the two largest groups being mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ASCC). For ACC, having the best 5-year OS, it was 70 percent. Facial palsy, advanced stage disease, lymph node metastases worsened prognosis. ACC and polymorphous low grade carcinoma (PLGA) expressed c-myc and cyclin D1 to a larger extent than MEC.

In squamous cell carcinoma of the head and neck we examined the occurrence of Single Nucleotide polymorphism, SNP. We found that the SNPs in male and female patients differed from each other. In male patients the SNPs were associated with immune response while in female patients the association was to SNPs concerning inflammation. This means that different pathways were engaged in cancer development for men and women. We also found that the SNPs in patients were different from those expressed in the healthy controls.

Keywords: salivary gland carcinoma, adenoid cystic, mucoepidermoid, polymorphous low-grade carinoma, c-myc, cyclin D1, perineural

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“Life is what happens to you while you are busy making other plans”

John Lennon: Beautiful Boy (Darling Boy, Double fantasy, 1980)

To my wonderful daughters Matilda,
Magdalena, Johanna and Olivia

List of Papers

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

I Sun, A. Sorensen, J. Karlsson, M., Turesson, I. Langstrom, B., Nilsson, P., **Cederblad, L.** Bertling, J, Riklund, K., Johansson, S. (2007). 1-[11C]-acetate PET imaging in head and neckcancer--a comparison with 18F-FDG-PET: implications for staging and radiotherapy planning. *Eur J Nucl Med Mol Imaging,* 34(5): 651-7

II **Cederblad, L**., Johansson, S., Enblad, G., Engstrom, M., Blomquist, E. Cancer of the parotid gland; long-term follow-up. A single centre experience on recurrence and sur vival. *Acta Oncol 48(4):549-55.*

III **Cederblad, L.** Thunberg, U. Engstrom, M. Castro, J. Rutqvist, L.-E. Laytragoon-Lewin, N. The combined effects of single nucleotide polymorphisms, tobacco products, and ethanol on normal resting blood mononuclear cells. *Nicotine Tob Res. 15(5): 890-5.*

IV Laytragoon-Lewin, N, **Cederblad, L**, Andersson, B-Å, Olin, M, Nilsson, M, Rutqvist, L-E, Lundgren, J, Engström, M, Tytor, W, Löfgren, S and Lewin F. Single Nucleotide Polymorphism and Cancer Risk, Tumour Recurrence or Survival of Head & Neck Cancer Patients. *Oncology.2017;92(3): 161-169.*

V **Cederblad, L**, van der Wal, J, Nylander, K, and Laurell, G. Expression of Cyclin D1 and c-myc in adenoid cystic carcinoma mucoepidermoid carcinoma and polymorphous low grade carcinoma. *Manuscript*

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Contents

Svensk sammanfattning

Huvud- och halscancer (HHC) är den sjätte vanligaste cancerformen i världen. I de nordiska länderna med något lägre incidens är HHC den tionde vanligaste cancerformen. Behandling av HHC är oftast en kombination av kirurgi och strålbehandling, men tillägg av cytostatika eller antikroppar förekommer relativt ofta och kan förbättra överlevnaden hos subgrupper av patienter. Nya målsökande läkemedel är substanser som med framgång använts inom andra tumörgrupper och som nu undersökts i studier av HHC. Dessa nya preparat är riktade mot proteiner i cellmembranet, Programmed cell death protein 1, PL1, och Programmed cell death protein ligand 1, PD-L1, som fungerar som mål (targets) till de målsökande läkemedlen.

Studie I är en observationsstudie med den långsiktiga målsättningen att kunna utveckla bättre metoder för att behandla HHC och dess metastaser. Syftet med radioterapi är att rikta den mot tumören med marginal och undvika strålning mot riskorganen (OAR) så mycket som möjligt för att minska komplikationerna som uppstår vid radioterapi av HHC. FDG-PET/CT jämfördes med ACE-PET/CT varvid vi kunde identifiera att tumörvolymen vid ACE-PET/CT var 51 % större samt att ACE-PET/CT fann flera tumörmanifestationer, såväl primärtumör som metastaser, jämfört med FDG-PET/CT. Dock kan man ifrågasätta om den större volymen vid ACE-PET/CT delvis berodde på lipidmetabolismen, vilket i så fall gav en falsk volymsökning.

Arbete II, är en retrospektiv studie av patienter med parotiscancer behandlade på Akademiska sjukhuset och med diagnos mellan åren 1948-2004. Överlevnaden var densamma under de två studerade perioderna, 1970-tal jämfört med 1990-tal. Faktorer såsom stadium, perineural växt, ansiktsförlamning och hög ålder identifierades och misstänktes bidra till försämrad prognos och överlevnad hos patienterna. Fyrtio procent av patienterna fick återfall. Fem-årsöverlevnaden var 53 %. På grund av de fynd som gjordes i arbete II undersöktes spottkörtelcancer ytterligare i arbete V. I arbete V jämförde vi tre undergrupper av spottkörtelcancer: adenoidcystic cancer (ACC), mucoepidermoid cancer (MEC) och polymorphous low grade adenocarcinoma (PLGA). Vi fann att en stor majoritet (22/23) av ACC uppvisade högt uttryck av c-myc. Tumörer i ACC hade också ett högt uttryck av cyclin D1. PLGA hade också högt uttryck av c-myc men inte fullt så intensivt som ACC. När det gäller uttrycket av cyclin D1 var det också starkt för PLGA. MEC hade högt uttryck av cyclin D1 men lägre uttryck av c-myc.

För att finna ytterligare faktorer som kan bidra till att man väljer rätt behandling för den enskilda patienten har vi i två arbeten undersökt Single Nucleotide Polymorphism (SNP), vilket är varianter av DNA. I arbete III i som är en *in vitro* studie tog vi blod från friska frivilliga kontrollpersoner. Ur kontrollernas blod extraherades vita blodkroppar som vi sedan exponerade för etanol, tobaksrök, snus och nikotin. I dessa vita blodkroppar undersökte vi hur varianter av arvsanlaget (single nucleotide polymorphism, SNP) reagerade när vi exponerade blodkropparna för de olika ämnen vi hade valt. Arbete III och IV är också att betrakta som screening av blodet hos friska kontrollpersoner och patienter med skivepitelcancer i huvud- och halsområdet för att hitta de SNPs som kan vara intressanta för utveckling av HHC. Vi undersökte hur många procent av cellerna som överlevda efter tre dagars behandling med de utvalda ämnena.

Celler i kroppen vandrar genom cellcykeln och skadade celler sorteras bort. Om cellerna utsätts för toxiska ämnen som stör denna process i cellcykel förändras arvsanlaget (så kallad mutation), och risken är att cellerna skadas så att det sker en utveckling mot cancer. Vi fann att det förekom olika SNPs hos män och kvinnor. Delvis skulle detta kunna förklara det faktum att en klar majoritet av patienterna som utvecklar cancer i huvud- och halsområdet är män.

Bidragande till skillnader i incidens är också att det historiskt är fler män än kvinnor som har varit rökare. För män är det mest vanligt med SNPs som förknippas med inflammation medans det för kvinnor är de SNPs som förknippas med immunologi. Utifrån våra resultat kan man spekulera i att vissa SNPs till del kan förklara varför inte alla rökare får cancer i huvud- och halsområdet.

Abbreviations

ACC	Adenoid cystic carcinoma
ACE-PET	^{11}C-Acetat-PET
CHT	Chemotherapy
CRT	Chemoradiotherapy
CT	Computed tomography
CTV	Clinical target volume
DNA	Deoxyribonucleic acid
dsDNA	Double strand DNA
EGFR	Epidermal growth factor receptor
EBV	Epstein - Barr virus
ENT	Ear, Nose and Throat
ERB	Ethical review board
FDG-PET	^{18}F-fluoro-2-deoxy-D-glucose (FDG)-PET
GTV	Gross Tumour Volume
Gy	Gray
HHC	Huvud- och halscancer
HN	Head and neck area
HNC	Head and neck cancer
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
IMRT	Intensity modulated radiotherapy
MEC	Mucoepidermoid carcinoma
MRI	Magnetic resonance imaging
M-stage	Stage of distant metastases (M)
N-stage	Nodal status (N)
OAR	Organ(s) at risk
PBMC	Peripheral blood mononuclear cells
PD1	Programmed cell death protein 1
PD-L1	Programmed cell death protein ligand 1
PET	Positrion emission tomography
PET/CT	PET and CT made at the same time for better anatomical definition
PLGA	Polymorphous low-grade adenocarcinoma
PTV	Planning target volume
RNA	Ribonucleic acid
SCC	Squamous cell carcinoma
SIB	Simultaneous integrated boost

SNP	Single Nucleotide Polymorphism
ssDNA	Single strand DNA
SUV	Standard Uptake Value
T-stage	Stage of primary tumour (T)
VMAT	Volumetric Arc Therapy
3D	Three dimensional

Introduction

Head and neck cancer

Human cancers comprise of a large variety of diseases with varying grade of aggressiveness. Today there are over 200 different types of cancer. The group of malignant tumours known as head and neck cancer (HNC) is quite common and is the sixth most common cancer worldwide (1). In the Nordic countries HNC is a relatively uncommon cancer, being the tenth most common cancer with approximately 1500 cases per year in Sweden (2). The global trend is the same as in Sweden with a growing number of cases attributed to an ageing population (3, 4). HNC comprise a large array of different tumours located in the head and neck area. Most of these cancers come from the mucosa, approximately 90 % are squamous cell carcinoma (SCC) (5) but other histopathology types exist such as adenocarcinomas (6) and several types of histopathology in the salivary glands (7). In earlier World Health Organisation (WHO) classifications many tumours of the salivary glands were classified as adenocarcinomas. As more data that was gathered, more groups of distinct entities were described and it became obvious that adenocarcinomas was a much smaller group than was believed earlier (8).

There is a gender difference in the HNC diagnosis. A majority or approximately 80 % of the patients are male (9). However, location of the tumour seems also to be gender dependent since more males have cancer of the larynx and hypopharynx and a larger proportion of females have salivary gland tumours (10, 11).

Today the approach for treatment of HNC is indisputably that of a multidisciplinary one, when planning the treatment of HNC (12). To treat HNC, certain factors need to be addressed. The startup when planning treatment of HNC is physical examination, biopsy of the lesion, some form of imaging such as CT, MRI, PET/CT and/or ultrasound guided fine needle aspiration. The data gathered from these examinations is considered together with the information on the patient's status and a decision of possible treatment is made. The location of the primary tumour affects the possibility to perform surgery. HNCs are situated in an area of the body with many important and life sustaining functions. It is therefore important to have as clear a view as possible of the extent of the tumours. If the tumour is accessible for surgery the next step will be to decide if the treatment needs to be combined with radiotherapy and if the radiotherapy needs the addition of

chemotherapy. The newly introduced "Standardiserat vårdförlopp" (Standardized treatment course, Socialstyrelsen, The National Board of Health and Welfare, 2015) in Sweden, has further improved the understanding of treatment for HNC and the swiftness that is required, to achieve cure. It is important to start treatment within 4 weeks from diagnosis as shown in earlier studies (13-15). For the outcome of HNC early discovery is important as is a precise description as possible of the extent of the tumour. Several studies have shown that spread to regional lymph nodes of the neck impairs the outcome and worsens the prognosis (16-18). Furthermore, attempts have been made to improve the outcome after radiotherapy or chemoradiotherapy with the help of CT or PET scan evaluation (19, 20).

In Sweden in 2015, the patients diagnosed with HNC had 5-years survival of 67 % and were considered cured according to the Swedish National Board of Health and Welfare, annual report for 2015 (Socialstyrelsen, Öppna jämförelser, 2015). These results stand well in an international comparison (21-23).

Approximately 2/3 of the patients diagnosed with HNC are male, thus representing the majority of the diagnosed cases. Females diagnosed with HNC are more prone to have salivary gland tumours. Most patients who are diagnosed with HNC are elderly, here defined as older than 60 years of age. Since the cohort of HNC patients is an ageing population, co-morbidity is common and has been shown to have a worsening impact on outcome (24). However, specific studies on elderly patients having HNC have not been performed to the same extent as in younger patients. Elderly patients being in good condition and without much co-morbidity may be able to go through a curative treatment for HNC and in selected cases chemoradiotherapy (CRT) might be possible despite the larger strain this will be on the patient. Traditionally, the addition of chemotherapy (CHT) to RT has been proven to increase local control and cure. The biggest advantage is the concomitant use during RT for stage III and IV disease (22). The use of neoadjuvant CHT, before any other treatment, has only been proven to be advantageous in nasopharyngeal carcinoma due to co-morbidity (24).

When it comes to treat patients older than 60 years, it was shown in a study by Jilani and coworkers that there was no survival advantage for elderly patients when using CRT (25, 26). In some studies, the survival has been worse for patients over 60 years when receiving CRT as compared to RT. This was because elderly patients were more prone to infections and other treatment related complications (27-29).

In recent years there have also been an interest for Programmed cell death protein 1, PD1 and Programmed death-ligand 1 (PD-L1) and blockage of these targets, since immunotherapy have been proven useful in the treatment of malignant melanoma and pulmonary cancers (30-32).

Epidemiology and incidence

The most common cancers of the head and neck in Sweden are cancers of the oral cavity, circa 350 cases (approximately 24 %), the oropharynx, circa 325 cases (approximately 22 %) and the larynx, circa 180 cases (approximately 12 %) all data per year (2). Cancer of the salivary glands is the fourth most common HNC in Sweden with an incidence of circa 100 new cases (6-7 %) per year. However lip cancer is about the same number but the data concerning lip cancer is ambiguous because there are many physicians in different specialties dealing with lip cancer: EarNose-Throat (ENT)-surgeons, plastic surgeons and general practitioners (2).

The fastest growing group in the upper aero-digestive tract is cancer of the oropharynx. This increase is attributed to a growing incidence of cancers associated to human papilloma virus (HPV), especially subgroup 16, which also is well known to be associated to female gynaecological cancer of the cervix (33, 34).

The total number of HNC does not include cancer of the thyroid gland which is considered a cancer of the head and neck region in many countries. However, in Sweden it is not included in HNC since thyroid gland cancer is endocrine by origin not emanating from the mucosa or salivary glands of the head and neck.

Etiology and risk factors

In some studies there have been suggestions that there might be a hereditary component for HNC, since first grade relatives have been shown to have a higher risk to contract the same kind of HNC as the patient (35, 36). In the discussion of heredity, the interest have turned to variations of the DNA known as SNPs of which some have been shown to be associated with cancers in the upper aero-digestive tract (37, 38).

Delay of start of treatment has been demonstrated to affect outcome. A study in Denmark compared start times in 1992 to those in 2002, in which the time to treatment start had been lengthened. A 10 % lower tumour control probability was attributed to much more examinations being performed before treatment start in 2002 compared to 1992 (39).

Risk factors known to be important for development of head and neck cancers are the use of tobacco, alcohol, infections from subtypes of human papilloma virus (HPV) for oropharyngeal cancer (40, 41) and Epstein-Barr virus (EBV) for nasopharyngeal cancer (42, 43).

In some parts of the world where betel chewing is common, this is also associated with the development of SCC of the HN primarily in the oral cavity (44). There has also been much attention on environmental factors, but this is still under debate to what impact it may have for the development of HNC.

Thus far, no conclusive findings, apart from the ones discussed above, have been presented (44, 45).

As mentioned above, HPV infections contribute to the development of cancer of the oropharynx, i.e. tonsillar cancer and cancer of the base of the tongue (46, 47). HPV is a large group of viruses that first were identified in the early 1980's by Syrjanen and co-workers as being contributing to development of condylomas of the cervix in females (48). During the 1980's it was also understood that HPV infections did play a role in development of pre-stages and cancer development of the cervix (49). It was later noted that HNC could be associated with HPV infection and it has also been shown that patients with HPV-16 infection had a better prognosis than patients contracting their cancer through smoking and heavy drinking (50-53). This leads to the suggestion that HPV dependent squamous cell carcinoma of oropharynx is a separate entity with better prognosis than the traditional" HNC (19). There are now well over a hundred HPV classes known to man but only 13 known to be associated to cancer development (https://www.cdc.gov/hpv/parents/whatishpv.html).

Diagnoses

For many patients the first sign of disease is a lump on the neck. This lump is investigated with cytology or core needle biopsy to establish the origin of the lump (54, 55). To continue the investigation some sort of radiological investigation is performed. All this leads to a diagnoses and this makes decision of treatment for the patient possible. It is also possible to use FDG-PET/CT (combination of FDG-PET and computed tomography). The extent of the disease with known primary tumour and known lymph node engagement is vital for the decision of treatment. Lymph node metastases are relatively common and results in poorer prognosis for the patient (56, 57). Distant metastases at primary diagnosis is uncommon and only in 6 -10 % of primary head and neck cancer has the tumour spread to distant locations at diagnosis (58-61). The prognosis is very much poorer for this group of patients and cure is not possible.

There are several studies showing that delay between time of diagnosis and start of treatment worsens the prognosis and patient outcome considerably (13, 62, 63). It is therefore of uttermost importance that patients seek help and that the doctor responds quickly in suspected cases of HNC.

The necessary swiftness in handling cases with suspected cancer in the head and neck region is nowadays well know, but there are still unfortunate cases with delays in treatment start. It is important that the start-up includes a multidisciplinary conference to optimize the handling of the patient and the disease (Socialstyrelsen, Standardiserat vårdförlopp 2015).

Squamous cell carcinoma

Squamous cell carcinoma is the absolute majority of cases and constitutes approximately 85-90 % of all HNC's. The tumours arise in different locations of the mucosa in the upper aero-digestive tract, such as the nasopharynx, the oropharynx, the oral cavity, the hypopharynx and the larynx. Depending on what location the tumours arise in, the prognosis varies, and most often males are afflicted. As previously stated, alcohol and smoking are the main causes of these cancers and of late HPV has been identified as a contributing factor mainly in younger patients without a history of smoking and drinking heavily. The male dominance in the patient group contracting these diseases has a historical explanation, since it was more common for men to be smokers in the 1900's and female smoking was not as common as it is today. Further evidence for the connection is that the tumours tend to appear in the area were the tobacco smoke passes. The prognosis for HNC has been poor for the tumours that are connected to alcohol and tobacco. The HPV dependent tumours have a much better prognosis and answer much more readily to treatment. The most common site of spread is to the adjacent, regional lymph nodes of the neck. In locally advanced cases, however, there is the risk of spreading to distant sites like the lung or liver. In cases with distant spread there is no possibility to achieve cure, but long-time survival might be possible in some cases (64).

Salivary gland tumours

Salivary gland tumours are a group of tumours originating from the cells of the salivary glands of the head and neck. Anatomically salivary glands are divided into major and minor salivary glands.

The major salivary glands consist of paired organs of the parotid, the submandibular and the sublingual glands. The minor salivary glands are all the other salivary glands which are spread out in the mucosa of the head and neck. All salivary glands help to moistures the mucosa and help to produce the saliva of the mouth and prevent dryness in the oral cavity.

Tumours of the salivary glands are a group of mixed composition. According to WHO there are 24 subgroups (65). This explains the complexity of the group and why it is so complicated to perform prospective, randomised trials in a large cohort of patients with salivary cancer. This also gives a hint as to why most studies on salivary gland tumours are retrospective and with a limited number of participants in each study (66-68). One randomised trial on different radiotherapy technics is on-going (66).

In the literature overall-survival at 5 and 10 years was 37% and 20%, respectively, the 5-year overall survival ranges between 37 and 55 % (69-71). The two most common malignant salivary gland tumours are the

histopathological types of adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC) (72).

Treatment for salivary gland tumours has traditionally been surgery, but in later years there has been support for that some salivary gland tumours seems to benefit of the addition of postoperative radiotherapy. Chemotherapy, however, has not been shown to make an impact on survival in spite of several trials through the years (73-75). Mucoepidermoid cancer (MEC) is divided into low, intermediate or high grade. The grading system reflects the prognosis with much poorer prognosis for high grade tumours (76). Intermediate and high grade MEC are also very intriguing and needs to be addressed to try to establish more treatment options for these cancers.

Cancer of the parotid gland has traditionally been treated with surgery with or without the addition of radiotherapy (7). The treatment has not changed markedly since the 1970s apart from an attempt by Wang and colleagues to use hyperfraction as a means to improve treatment outcome for patients with cancer of the parotid gland (77).

However, for some types of salivary gland tumours surgery with the addition of radiotherapy does not seem to be sufficient. This concerns for instance adenoid cystic carcinomas (ACC), which is a tumour type that readily spreads along nerves (78-80). This sub-group of salivary gland cancer have not the worse outcome but needs to be treated with radiotherapy after surgery to improve on outcome.

Treatment

To treat head and neck cancer, several factors needs to be addressed. As mentioned earlier, the decision of the optimal treatment is made by accounting for the extent of the tumour, the presence or absence of lymph node metastases and the histopathology of the tumour. Nowadays it is mandatory to involve a multidisciplinary conference with Ear-Throat-Nose (ENT) surgeons, oncologists, radiologists and pathologists. Several other specialists may also be involved depending on what treatment is considered necessary. It is also important to consider potential patient co-morbidity. Many of the patients are elderly, which in these circumstances is considered to be older than 60 years. However, it is not the age in itself but the co-morbidity that may led to that the patient is unable to cope with the tough treatment that could cure them. Thus, to try to find the most optimal treatment for the patient, the age and co-morbidity is necessary to be considered (27, 81, 82).

Surgery and Radiotherapy

In many cases of HNC, surgery is a large part of treatment. The ideal situation is that surgery is radical with good margin (preferably at least 1mm) between

the tumour(s) and the resection edge. Surgery and radiotherapy are the two most important treatment modalities for HNC. Combinations of these two treatment modalities are often the case if the goal is to achieve cure for HNC patients. In cases of surgery, radical results are important. Extension to resection margins has been proven to be an aggravating factor for outcome of cancer disease (83). When it is not possible to achieve radical surgery, the addition of radiotherapy is of vital importance. In some cases, curing the patient will not be possible. These cases include when the patient is very old, have aggravating co-morbidities, or have a tumour that has spread to distant locations in which case cure is not achievable. Then best supportive care, palliative surgery, or radiotherapy can be performed with the clear goal to mitigate symptoms for the patient and improve quality of life (84, 85).

However, still the most optimal treatment approach is the combination of both surgery and radiotherapy. The combination of surgery and radiotherapy possesses an advantage compared to single treatment modality. The combination has been proven to be the most optimal treatment option for treating cancer of the head and neck region. Even though organ sparing techniques has become more commonplace, in many cases surgery still needs to be part of the treatment. It is clear the radiotherapy has gained in importance and contribute to give improved treatment results for patients with HNC. In some cases, it is on the contrary radiotherapy that is the treatment to start with. This is true for instance for laryngeal cancer or cancer of the base of the tongue.

As early as 1899, the first successful curative treatment with radiotherapy of a tumour, a basalioma of the skin, was performed, merely four years after the ground breaking event of discovering radiation by Wilhelm Conrad von Röntgen which eventually gave him the first Nobel Prize in physics in 1901 (86). The third Nobel Prize in physics was awarded to Marie Curie, her husband Pierre, and Henri Becquerel in 1903 for their research on radioactivity, which also has a place in cancer treatment. Since then, a long line of discoveries and experiments has led to the treatment options for radiotherapy that we have today.

Single modality treatment with radiotherapy can be given as curative or palliative treatment. For radiotherapy, it has been widespread practice to perform one fraction of 2 Gy per day, 5 fractions per week, for 7 weeks to the total dose of 68-70 Gy. During a period from the early 1970s through the 1980s many institutes used split course treatment with a pause after approximately 40 Gy (20 fractions) (87). The time for the pause corresponded to circa 4 weeks of treatment and coincided with the skin erythema that appeared around this time in treatment. The treatment outcome was in some studies unaltered but in other cases it became apparent that the introducing of a pause led to worsened outcome for the patients (88). For this reason, other studies were performed where shortened treatment time without pause was introduced. The CHART-study by Saunders and co-workers from 1990 was

such a study showing the importance of shortening the treatment time and avoid pauses in the treatment (89). Their model of treatment is not possible to introduce on a larger scale since they gave a fraction every eighth hour and completed the whole treatment in twelve days which is very resource consuming. This kind of resources are not available outside a study.

In later years attempts to increase the dose to tumour manifestations has led to the development of intensity modulated radiotherapy (IMRT). IMRT was originally designed to produce higher dose to target and at the same time avoiding irradiating organs at risk (OAR) particularly the parotid glands (90, 91). However, the surrounding non-target tissue receives also a high dose when IMRT is used. To be able to increase the dose to target even higher, towards 80 Gy, without raising the dose to non-target tissue, the technique of simultaneous integrated boost (SIB) was developed (92, 93). Further development of the IMRT has been achieved with the use of volumetric arch therapy (VMAT). The radiotherapy is a seven-week long treatment period when it comes to curative or adjuvant radiotherapy and vitiated with challenging side effects, acute radiation reactions that impair the patients' ability to eat, drink and withstand pain which can be quite intense and demands a close contact with weekly treatment rounds and monitoring of the patients. In most cases the use of morphine is mandatory. However, late side effects of the treatment, from both surgery and radiotherapy are also demanding for the patients with disabilities of various grades especially in chewing, swallowing and speech. The more advanced the tumour is the more demanding the treatment becomes.

Chemotherapy

It is known that chemotherapy (CHT) does not cure HNC, but can be a good addition to radiotherapy in CRT. For some tumours the most optimal approach is to be given CHT once a week concomitantly with radiotherapy. This concerns for example tonsillar cancer and cancer of the base of the tongue improving outcome in fit patients (94). The breakthrough for CHT came when the use of concomitant CHT was added. It is mostly platinum based CHT that is being used once a week and this has a good effect on treatment outcome. There is the use of CHT in the palliative setting to mitigate symptoms and hopefully improve the time left for the patient. In the beginning full cures were given with a combination of the CHTs, most often cisplatinum and 5-FU every third week during RT. This proved to be too toxic for most patients and their bone marrow. It was when the use of single agent treatment once a week was being introduced that the advantage of concomitant use with cisplatin was established. Few other CHT has shown the same advantage.

Antibodies

The introduction of antibodies into the treatment arsenal was a result of the finding that approximately 85-90 % of squamous cell carcinoma of the head and neck (SCC) expresses epidermal growth factor receptor, EGFR (95-98). The option of adding antibodies directed towards EGFR opened for new treatment combinations, particularly in advanced cases. The epidermal growth factor (EGF) and the receptor (EGFR) were discovered by Stanley Cohen at the Vanderbilt University USA and led to a Nobel Prize in Medicine in 1986 (awarded together with Rita LeviMontalcini). The EFGR is a transmembrane protein that is a receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands. Research has shown that there is an overexpression of the receptor in several human cancers of which squamous cell carcinoma of the head and neck is one. The overactivity led to increased growth of the cancer cells.

This growth can be halted using antibodies aimed at the receptor and this hinders the further development of the cancer. The first paper published on this subject in HNC was by Bonner et al in 2006 (99) who could prove that patients with EGFR overexpression had better treatment outcome if they were given the antibody against the receptor. However, this study has been greatly criticized. There are a lot of cutaneous negative reactions during this treatment but Bonner and others have continued to use cetuximab in trials to try to find a place for it in the treatment arsenal (100). The discovery and use of EGFR was confirming that molecular therapy may be a way forward towards more treatment options for HNC hopefully improving outcome (101).

A new treatment option has recently emerged, with the addition of programmed cell death protein-1, PD-l, and programmed cell death ligand-1, PD-L1, antagonists which in some studies have proved to result in promising outcomes especially in carcinoma of the lungs and malignant melanoma. This has broadened the treatment arsenal for these two tumour types. The hope is now that HNSCC tumours will benefit in the same way from this treatment (102). PD1 is a cell surface receptor that plays an important role in down-regulating the immune system and functions through suppressing T-cell inflammatory activity. PD-L1 is a transmembrane proteins thought to play a major role in suppressing the immune system during specific event for instance tissue allografts, autoimmune disease and other diseases. Today studies are on-going in patients with HNC and preliminary results have been showing promising results (102-105). Both preclinical studies and now on-going clinical trials are continually showing intriguing findings (106-108).

Purpose and aims

The overall aim of the thesis was to identify factors for treatment response and survival in mixed cohorts of patients with head and neck cancer.

The specific aims of the individual papers were:

Paper I: An observational study.
The aim of paper I was to evaluate the feasibility of using 1-[^{11}C]-acetate positron emission tomography (ACE-PET) to detect and delineate the Gross Tumour Volume, GTV, of HNC before radiotherapy, and to compare the results with the results using ^{18}F-fluoro-2-deoxy-D-glucose (FDG) PET.

Paper II: A retrospective study.
The aim of this study was to investigate the results of treatment of cancer of the parotid gland,focusing on tumour control and survival.

Paper III: An in vitro study.
This study investigated the putative impact of single nucleotide polymorphism (SNPs), ethanol and tobacco products on cell behaviour in blood from healthy individuals. This study also helped to screen for SNPs of interest when it came to cancer development of the head and neck and treatment outcome.

Paper IV: A study comparing 2 cohorts.
In this study the aim was to study the influence of SNPs on cancer risk, tumour recurrence and survival in HNC patients and compare the appearance of SNPs to controls.

Paper V: A retrospective study combined with immunohistochemistry.
The objective was to investigate a cohort of 48 patients with salivary gland carcinoma with special reference to the expression of c-myc and cyclin D1. Three types of tumours were examined, ACC, MEC and PLGA.

Ethics

All the studies were approved by the Ethical Review Board (ERB) in Uppsala and in studies with collaboration with other hospitals also by the local ERB as stated below.

Study I - ERB in Uppsala, 2009.

Study II - ERB in Uppsala 2005.

Study III and IV – ERB in Uppsala, 2006, also by ERB in Stockholm, 2009 and Linköping 2013.

Study V - ERB in Uppsala 2013 and ERB in Umeå 2015.

Material and Methods

Patient populations

Study I was a prospective observational study using two kinds of tracers performing PET/CT scans on ten consecutive, newly diagnosed squamous cell head and neck cancer patients. The purpose was to evaluate the feasibility to use ACE-PET/CT when planning for radiotherapy for HNC with a curative intent. Comparison was made to FDG-PET/CT which had been used in other hospitals with good results. The patients were collected from the Departments of Otolaryngology and Oncology at Uppsala University Hospital and from the Oncology department at Umeå University Hospital. As part of the study the dose planning images with ACE-PET/CT were performed and compared with the FDG-PET/CT. The PET scans with both tracers were performed before treatment start and the FDG-PET/CT scans were also performed twice during the radiotherapy treatment period in week 3 and 5 and finally circa 4 weeks after conclusion of the radiotherapy treatment. The standard up-take value, SUV, was estimated for each image sequence both manually and automated with the computer program.

Study II was a retrospective study on 144 patients with parotid gland cancer. The investigation period was 1948-2004 and based on records from the departments of Otolaryngology and Oncology in Uppsala. Many medical records had been microfilmed and were read through a microfilm recovery device. The data obtained regarded: age, TNM-stage, recurrence and treatment given. There was however little to no data on co-morbidity, use of ethanol and smoking. The overall survival was calculated.

In *study III*, an in vitro study, peripheral blood mononuclear cells (PBMCs) from 54 controls were studied. All the controls gave their consent to participate, answered questions on alcohol and smoking habits as well as donating 30 ml of blood from which the PBMC were extracted. In study III only blood from the controls was analyzed to present a picture of the normal appearance of SNPs in blood in a healthy population.

In *study IV* 174 patients with newly diagnosed squamous cell carcinoma of the head and neck area and bound for curative radiotherapy were recruited. In study IV we also recruited 245 controls since we recruited healthy blood donors from 3 more hospitals, Karolinska University Hospital, Linköping University Hospital and Jönköping Hospital. Patients and controls from study

III were all included in study IV. Both patients and controls donated 30 ml of blood, blood samples donated by patients and controls alike were analyzed.

In *study V* a retrospective study of 48 patients with salivary gland cancer were identified and surgical specimen collected from archives of the Pathology departments of Uppsala and Umeå University Hospitals were studied. The samples were archived material. We investigated the occurrence of c-myc and cyclin D1 in the samples to evaluate how many and to what extent it could be established.

The relevant clinical data for the patients in all the studies were obtain from medical records. Patients were staged according to the UICC TNM stage classification, 7th edition (109).

PET/CT

The positron emission tomography-computed tomography (PET/CT) is a machine that combines nuclear medicine technique (in a single gantry) PET scanner and an x-ray computed tomography (CT) scanner. This makes it possible, to in the same session, get sequential images from the PET and the CT, combined into a single superposed (co-registered) image. In the PET which is functional imaging you get the spatial distribution of metabolic or biochemical activity in the body. Doing the CTs at the same time makes it possible to correlate the PET to the anatomic imaging obtained by CT scanning. All the scans were performed in a fixation device according to routine. It was possible to achieve two- and three-dimensional image reconstructions due to the software program and the control system.

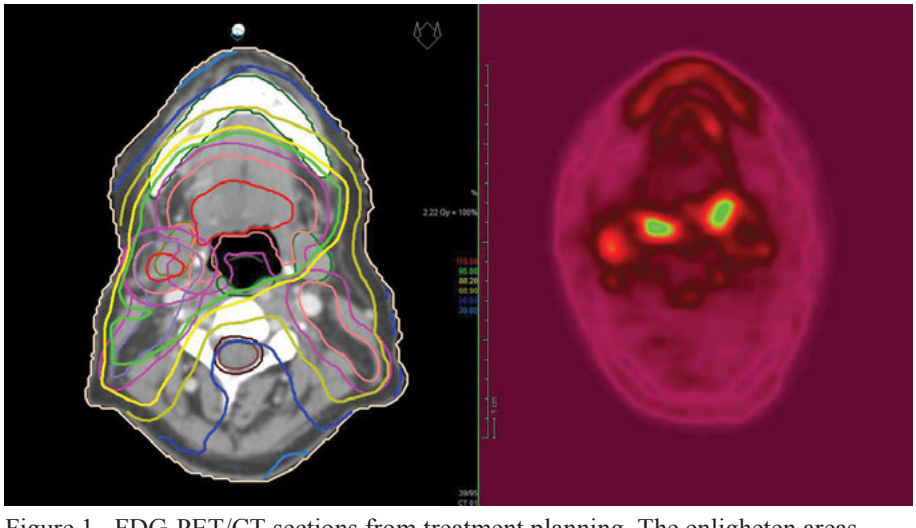

Figure 1. FDG-PET/CT sections from treatment planning. The enligheten areas represents the tumour and the metastases. Isodoses are

The way to evaluate PET images is to use Standard up-take value, SUV, which is a comparison between the up-take of the tracer in the lesions compared to the up-take value of the tracer in the rest of the body.

In paper I we used FDG-PET/CT and ACE-PET/CT to increase the possibility to clarify the extent of the tumour and detect metastases. We used a 32-min dynamic emission scan performed immediately after intravenous injection of 10 MBq/kg body weight ACE. In the next step we used intravenous injection of 5 MBq/kg body weight FDG.

Data collection

In study II the data collection was the backbone of the study. The medical charts were scrutinized for data on the tumour and its spread, treatment, survival and recurrence. Old age (> 60 years) was a factor which worsened outcome. This was because elderly patients were more prone to infections and other treatment related complications, survival and recurrence. Since some of the material was very old it was not possible to find all the pathological material. No examination of the material from surgery was performed.

Single Nucleotide Polymorphism, SNP

In study III and IV we investigated SNPs. SNPs are the most common type of genetic variation in humans. A SNP is a variation in a single DNA building block. This means that different SNPs have a nucleotide (adenosine, guanidine, thymidine, and cytosine) exchange made and have a replacement in one portion of a base-pair to form another pair. This leads to different SNPs that can have different effects on the human genome. Most SNPs, however, are silent and does not affect the genome. These variations occur between 100 and 300 base pairs apart in the whole genome (110).

The material collected in study III was blood from healthy controls and extracted as PBMC. The agents that the cells were exposed to were the Swedish snus prepared in an orbital shaker and then aliquot, protected from sunlight and stored at -80 C°, ethanol, nicotine and the cigarette smoke extracted was from filter cigarettes with a so called Borgwandt RM 20/CS-smoking machine. The particles in the filter were extracted with ethanol and concentrated using a rotary evaporator. The extracts were aliquot, protected from sunlight and stored at – 80 C°. The 0.2% ethanol and 100µM pure nicotine, snus extract or smoke extract contained 100 µM nicotine were freshly prepared before use in the test system. The cell cycle progression and cell death from these cell cultures were analyzed (111).

The analyses were performed in the SNP & SEQ technology platform at Uppsala University. One SNP (TP 53 codon72 (rs 1042522) in this study was

performed by PCR-RFLP (112). In study IV 45 SNPs located in 41 characterized, functional genes in blood from healthy controls and from patients with HNC newly diagnosed. High-molecular-weight genomic DNA was isolated using a standard automatic QIAGEN Bio Robot M48with MagAttract DNA blood kits. The quality and quantity of DNA was determined by the PICOGreen method. This is a method where the dsDNA is the only object that binds to the dye and in this process the dsDNA becomes fluorescent. PICoGreen is a proprietary asymmetrical cyanine dye, very sensitive method to distinguish dsDNA over ssDNA and RNA.

Histopathology and immunohistochemistry

Histopathology is a method to evaluate the anatomy of the tissue in the microscope. It is done by examining sliced samples of the tissue that are mounted on glasses for microscopy, stained with suitable dye and examined in a light microscope. Immunohistochemistry (IHC) is a method of selectively detecting antigens that most often are proteins, in cells of a tissue section by using antibodies binding specifically to the antigen in biological tissues (biopsies). This is widely used in the clinic and in research to find abnormal cells for example cancer cells in tumours. To distinguish specific cells different molecular markers can be used.

Results

Paper I

The study showed that the addition of 1-[^{11}C]-acetate and ^{18}F-fluoro-2-deoxy-D-glucose PET gave a more detailed picture of the spread of the tumour compared to CT alone. This meant that both ^{18}F-fluoro-2-deoxy-D-glucose - and 1-[^{11}C]-acetate-PET/CT improved the survey of the tumour for the primary tumour a well as for the regional metastases. Both quantitative and semi-quantitative estimates improved the detection of tumour spread.

There was a discrepancy between the up-take of ACE compared to the uptake of the FDG. All of the primary tumours (10/10) were detected by ACE-PET, while only nine were detected by FDG-PET and CT or MRI. ACE-PET was better at detecting the metastases but failed in one case to detect this metastases due to a very small volume. This means that ACE-PET/CT detected 20/21 metastases. FDG-PET/CT detected 13/21 metastases. The patients had all done either CT or MRI which were the investigations that were used as the role model for tumour detection. The SUV was estimated.

Paper II

The five-year overall survival for the entire group was 53 %. Treatment was surgery with or without adjuvant radiotherapy. For younger patients (<65 years of age) the five-year survival was 58 % and for older patients it was 23 %, $p<0.00001$.

The best 5-year survival of 70 % was reported in patients with adenoid cystic cancer. Patients with mucoepidermoid carcinoma had the poorest prognosis with a 5-year survival of 30 %. However, no distinction was made between high, intermediate or low grade mucoepidermoid tumours since this division was not done at the time.

For patients with facial palsy at diagnosis (14 % of the patients in this material), the survival was poorer than for patients without facial palsy with a survival at 5-year of 18 % compared to 55 % ($p=0.017$). As could be expected, patients with a more advanced stage had a poorer prognosis than those with earlier stages. Patients with stage I disease had a 5-year survival of 59 %, stage II 48 %, stage III 37 % and stage IV 22 % respectively. The differences are significant ($p=0.0094$).

Patients with lymph nodes metastases had worse prognosis than patients without (5-year survival for N+ 15 % versus N0-stage 75 %). Fifty-two patients (36 %) died from parotid gland carcinoma and another 15 % died of other cancers. In a comparison between the 1970's and the 1990's no significant difference in treatment and overall survival (p=0.475) could be shown even though the median radiation dose to the target was slightly higher in the 1990's in comparison to the 1970's (59.9 Gy versus 62.2 Gy). Time to recurrence after termination of treatment was 1.4 years in median and 3.2 years as a mean. The use of split course did not significantly affect survival.

Paper III

In paper III we extracted blood from a healthy population and screened the blood from the donors to see what SNPs were present in the blood in a population who had not been diagnosed with a malignancy. We investigated 30 candidate genes and 10 of these had a relationship to the cell cycle. The SNPs correlating to the cell cycle progression were SNPs in the *IL12RB2*, *Rad 52, XRCC2*, *P53*, *CCND3* and *ABCA1* genes. Furthermore, when the PBMCs in the blood from the donors were exposed to EtOH, nicotine, smoke and snus in different combinations there was a substantial effect of induced cell death for SNPs in *Caspase 8*, *IL12RB2, Rad 52, MMP2* and *MDM2*. This reached significant influence from the agents in question. After three days of in vitro, the cells cultured were in G0/G1 stage in 93%, 2.6 % were in S stage and 1.8 % of cells in G2 stage for the control group. In the study where the PBMC were being subjected to ethanol, we observed that the PMBC proceeded to S-stage in the cell cycle after three days of the treatment. The highest degree of cell death, 19.8%, was observed in cells exposed to smoke + EtOH than the basal level in controls and other treatment conditions ($p<0.005$).

Paper IV

In study IV we expanded the material and studied SNPs from 174 newly diagnosed, H&N cancer patients of Caucasian origin and 245 healthy controls. The patients had all been diagnosed with histopathological verified squamous cell carcinoma of H&N cancer. Based on the data in the medical charts clinical staging was performed according to the WHO and UICC systems (109, 113, 114). The reference group in this study was the healthy controls from study III from Uppsala with an addition of healthy blood donors from Stockholm and Jönköping, in total 245 healthy individuals. There was a difference between males and females concerning the genes associated with cancer. The genes in the female patients with HNC were not the same as in the male

patients with HNC. The three SNPs in females that were linked to increased cancer risk were 1 SNP in *CCL5* and 2 SNPs in *PRF1*. For males, on the other hand it was 4 SNPs with increased cancer risk, 2 of the immune response genes (*TNFα* and *IL12RB2*), 1 SNP in growth factor gene *EGF* and 1 SNP in DNA repair gene *RAD52*. The observation period in the study was 36 months and during this period 59 (46 %) patients suffered recurrence (19 females [51 %] and 40 males [44 %]).

Patients with the genes *IL12RB2* rs3790568 AG and patients with and *IL12RB2* rs3790568 GG genotype died in 69 % and 40 %, respectively. The *IL12RB2* rs3790568 AG genotype patients had a significantly shorter survival time when compared to those with the GG genotype ($p = 0.006$). The highest proportion of deaths, 11 out of 14 (79 %), was observed in the *TNFα* rs1800629 AA+AG phenotypes. A lower proportion, 45 out of 114 (39 %), was observed in the patients with the *TNFα* rs1800629 GG genotype. The *TNFα* rs1800629 AA+AG genotype patients had a shorter survival time when compared to those with the GG genotype ($p = 0.001$).

Paper V

In study V we investigated the expression of c-myc and cyclin D1in three entities of salivary gland tumours, ACC, MEC and PLGA from tumours from 48 patients. ACC and PLGA come from the same kind of cells but ACC is a more aggressive cancer. In ACC and PLGA it can sometimes be difficult to separate one from the other.

In ACC we found that 97 % of the tumours had high levels of c-myc, 87 % had high levels of cyclin D1. PLGA, and 83 % (5/6) of the tumours had high levels of both c-myc and cyclin D1. In MEC, 37.0 % had high level of c-myc, and 79 % had high level of cyclin D1. This means that c-myc was highly expressed in both ACC and PLGA but here the intensity was lower in PLGA. The overall 5-year survival was 69.9% in total. For ACC, MEC, and PLGA, the 5-year survival was 59, 82, and 83 % respectively. As expected, 5-year survival was much better in low stage (I and II) 79 % compared to the high stage tumours (stage III and IV) which had a 5-year survival of 50 %. Perineural spread did not influence the outcome in this material.

Discussion

PET/CT

When planning radiotherapy regardless of tumour type the use of CT in some kind of fixation has been standard since the 1980ies in the Nordic countries (115).

In many radiotherapy departments around 2005/2006, FDG-PET was also accepted as a complementary tool in treatment planning. An important consideration when making plan for radiotherapy is that the patient is in the same position every time during the treatment. To achieve this HNC patients are positioned in a fixation device. In study I (conducted between 2005 and 2006) FDGPET/CT and ACE-PET/CT were both beneficial in determining radiation planning for HNC. As a result of the study, we have since then used FDG/PET-CT with the patient in a fixation device for planning radiotherapy. The fixation device was also used before using the PET/CTs, but the combination of fixation device and PET/CT now enables us to diminish the margins from Gross tumour Volume, GTV, to clinical target volume, CTV, due to more precise delineation of the tumours. GTV is the tumour manifestation of the primary tumour and GTVN for the lymph node (N) metastases. The larger volume presented by the ACE-PET/CT can possibly be attributed to the lipid metabolism which has been demonstrated by Yoshimoto (116). The use of FDG-PET/CT now helps us to achieve a high accuracy in treatment planning. The reason for using FDG-PET/CT and not ACE-PET/CT at Department of Oncology today is twofold: FDG-PET/CT was more accessible since it was easier to produce the tracer and the insecurity of the larger volume being partly due to lipid metabolism made us hesitant to use ACE-PET/CT in HNC planning. Several other hospitals also used FDG-PET/CT for different tumour types with good results (117, 118). However, a crucual finding in study I was that ACE-PET/CT was better at detecting tumours and metastases than the FDG-PET/CT. SUV was declining after treatment as a measure that the treatment was effective. We also noted that the SUV values were declining during the radiotherapy, which we followed through repeated FDG-PET/CT during the treatment period.

The idea to narrow the margins around targets may diminish the effect on OARs which in turn diminishes the strain and side effects resulting in less acute and long-term problems for the patients with HNC after radiotherapy. The introduction of Intensity modulated radiotherapy,

IMRT, and later the introduction of VMAT, Volumetric Arc Therapy, has further improved the narrowing target margins using FDG-PET/CT in radiotherapy planning which has been beneficial for HNC patients with for instance better taste experience and less dryness of mouth and therefore better mouth hygiene (119, 120).

Salivary gland cancer

Salivary gland tumours are a complex entity, being uncommon and having many sub-classes, which makes it difficult to perform randomised trials. Salivary gland cancer of the parotid gland and other major salivary gland cancers are till this day treated with surgery with or without radiotherapy (7). In our study II on parotid gland cancer, the 5-year overall survival was 53 %. Overall survival has lingered around 50-55 % (71). Thanks to the introduction of IMRT some improvement have been made (121) according to a study by Zeidan et al from 2013 where overall survival was 76 % and 63 % at 5- and 10-years respectively (121).

In later years, more studies have indicated that other factors might influence the prognosis. In adenoid cystic carcinoma, for example nuclear RB1CC1 loss in the tumours has been proven to worsen the outcome for patients (122).

In paper V we investigated the expression of two other markers, c-myc and cyclin D1 which we investigated in three types of salivary gland tumours: ACC, MEC and PLGA. A characteristic feature was that a majority of ACC and PLGA tumours expressed c-myc to a larger extent than MEC. ACC and PLGA both also expressed cyclin D1 extensively. The high expression of c-myc for these two tumour groups might be of interest for future treatment options. If in the future it will be possible to target c-myc. However, these findings need to be confirmed in future studies. A large problem with ACC is loco-regional recurrence. In such case treatment options besides repeated surgery are scares although CHT have been attempted with dismal results only marginally improving the situation for the patients (123-126). For this reason, the addition of further treatment possibilities has been considered important. The interest has turned towards molecular targets and there are several recently published studies investigating molecular expression with the aim to find new treatment options (125, 127).

Single nucleotide polymorphism

The use of SNPs in this thesis was to find explanations for why some, but not all smokers develop cancer in the head and neck. The results in study III confirm that smoke and alcohol in combination worsens the effect on PBMC. These two agents gave rise to massive cell death, but did not show any direct influence on the cell cycle progression of human normal resting cells. SNPs might have a potential relevance in predicting risk individuals for developing

HNC in those individuals exposed to cigarette smoke and ethanol drinking. First-degree relatives to HNC patients are reported to have an increased risk of developing HNC indicating there may be an important role of genetic factors (35, 128). In paper IV we tried to further elucidate the effect of certain SNP sequence variations that may lead to altered effects of susceptible genes. These variations in SNPs may lead to and cause inter-individual differences that might influence on the susceptibility to risk factors and increase the risk to develop HNC. According to the findings in paper III, one may speculate that multiple pathways could influence the development of HNC cancer, pathways involving DNA repair, cell death, and immune response (129-132).

It is a well-established fact that a high proportion of HNC patients are middle-aged males (133-135), which was true in our study as well. Throughout history, males have been smoking more than females due to traditions in the society (136). It is well known that variations in the SNPs, result in altered effects of tumours and causes inter-individual differences that might influence the susceptibility to develop cancer (133, 137-139). This seems to confirm that multiple pathways are involved in DNA repair, cell death, and immune response and that may influence on the development of HNC as well. The alterations in the normal cell phenotype and gene expression profile has been attributed to toxic combustion products in cigarette smoke (rather than nicotine) (140). This event occurs in association with certain SNPs and is not random. It has been shown that HPV infection increases the inflammatory microenvironment (141), and productive HPV infection could also induce cell death (142). HPV or smoking induced cell death has been shown to be a consequence to chronic inflammation (141, 143, 144). Increased levels of inflammation-related proteins, CRP and pro-inflammatory cytokines, and TNFα in the plasma of have been found in HNC cancer patients (133).

As high-risk HPV type might be necessary for tumorigenesis in oropharyngeal cancer it cannot, however, be sufficient on its own (142). In most individuals HPV infection will trigger an effective immune response that clears all traces of the virus from the body (145). For females, an increased risk to develop HNC was only observed with SNP in the immune response gene *PFR1* and the chemokine gene *CCL5*. These specific genes have essential functions in cell migration and cellular-mediated cytolysis against viral infection (146, 147). It can be speculated that HPV infection might be more frequently associated with the development of HNC in females with specific SNP sequences in the *PFR1* and *CCL5* genes (146, 147). However, it is males that are in majority to develop oropharyngeal cancer. In males as, increased HNC risk was associated to SNPs in genes mediating DNA repair, such as *RAD52*, and genes coding for inflammatory cytokines such as *TNFα*, *IL12RB2*, *IFNG,* or the chemokines *CCL4* and *CCL5* (138, 148). This indicates that the risk of HNC among males might be more frequently associated with DNA damage and inflammation than among females.

Host immune response is essential for tumour control and the survival of cancer patients (149, 150). However, a relation between abnormal or residual tumour recurrence, survival, and SNPs of immune response genes detected in our investigation are supported by previous investigations (134, 138, 150, 151).

The SNPs are inborn stable factors, presumably independent, which seems to indicate that they have a role to play as genetic factors. Thus, there seems to be a correlation between inflammation and cell death that could explain why bearers of certain SNPs have an increased risk of inflammation which may drive the development towards the evolution to diseases such as cancer.

Our assumption was supported by increasing TNFα in the plasma of HNC patients. TNFα was found in patients with short survival in our study. CRP was not examined in this study but in other studies CRP findings have been made and indicated that inflammation is having an important role to play in the development of malignancy (152). In the treatment situation, the immune status of the patient is affected by radiotherapy initiating systemic inflammation which can be seen in blood as increased levels of interleukin 6 and CRP.

Since the *TNFα* rs1800629 was associated with HNC risk, tumour recurrence, and the clinical outcome there might be a possibility to use the SNP *TNFα* rs1800629 as a biomarker for individual treatment selection in the clinical setting of HNC. This could be an intriguing new possibility. There have been in vitro studies investigating this possibility (153, 154). But to our knowledge, no conclusive findings have yet been provided (153, 154). However, there is a study on periodontitis and cancer development in the head and neck presented by Shrihari, focusing on *TNF*α (154). But no clinical study has so far assessed *TNFα* as a biomarker for HNC or recurrence of HNC. This seems to be an area where development is possible and welcome. It might lead to future ways of diagnosing and eventually treating HNC.

Our results suggest that HNC are a group of malignant tumours associated with individual inherited genetic profiles in addition to the effects of established risk factors such as smoking, alcohol and HPV infection. Patients with a competent immune response might be able to control and inhibit development of cancer cells (149).

Conclusions

Head and neck cancer is a complex group of malignant tumours. The volume where the tumours arise is between the base of the skull to the clavicles. Each of the HNC has sub groups, the salivary gland tumours being the most obvious example.

To improve the outcome for patients with HNC where surgery and radiotherapy have been the mainstay of treatment for many years, new treatment options like the combination with chemotherapy and/or molecular therapies need to be addressed. This is a delicate task since many of the patients are older than 65 years and many cases have co-morbidities that are limiting factors. Therefore, some patients might not cope with the more advanced and demanding treatments because of their co-morbidities.

The treatment planning has been improved since the use of 3D-planning with CT has been introduced. After our PET/CT study with ACE and FDG we have used FDG-PET/CT, which have improved the quality of treatment planning at our department.

Another way to improve treatment for future patients is to examine SNPs which gave us the insight that men and women have different SNPs that may promote cancer development in the head and neck. The knowledge that different pathways are engaged in cancer development in men and women can explain the difference we experience in the clinic with larger fraction of patients being male. This may help us understand the mechanics behind cancer development and be a platform for future studies.

In this thesis we also investigated salivary gland cancer, ACC, MEC and PLGA with the longterm goal to identify factors that could be useful in search for novel ways to treat patients. The same goes for HNSCC where improved treatment with new targets such as PD1 and PD-L1 may be a way forward. The use of targeting SNPs could be another option for improved diagnostic tools. It may also offer a potential for development of new pharmacological therapies.

Future studies

Treatment for HNC seems to be at a cross roads. In several other tumour groups, like lung cancer and malignant melanoma, the use of molecular treatments and biological methods seems to be up and coming. The same

development is reasonable to expect in the area of HNC as well. In study III, IV, and V we investigated molecular markers. Future studies in this area for HNC are to be expected.

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