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SKIN CANCER IN RELATION TO TOBACCO USE AND ORGAN TRANSPLANTATION

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Till Mamma

1 ABSTRACT

Background: Skin cancer incidence in fair-skinned populations has increased more rapidly than any other cancer form during the past several decades. The possible impact of common exposures in the population, such as tobacco use and overweight/obesity, is not established.

The risk of cutaneous squamous cell carcinoma (CSCC) in organ transplant recipients (OTR) is substantially increased, although the underlying mechanism is not known. From previous studies of multi-drug regimens it has been suggested that risk of post-transplant CSCC is conferred by an overall immunosuppressive burden rather than by specific drugs.

Aims and methods: The first aim of this thesis was to evaluate the impact of tobacco use and body mass index (BMI) on the risk of CSCC and cutaneous malignant melanoma (CMM), melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM). To accomplish this aim we analyzed a large retrospective cohort of Swedish male construction workers (n≈ 340 000) with prospectively collected exposure information and virtually complete follow-up.

The second aim was to separate the effects on post-transplant CSCC occurrence of immunosuppressive level, infections, immunosuppressive drug use, and other potential risk factors. For this purpose we designed a case-control study, nested in the population-based cohort of OTRs (n≈ 6 000), and collected detailed exposure information from patient medical records by use of a standardized questionnaire.

Results: Current smokers were associated with a 30-50% risk reduction of CMM, MIS and IMM, compared to never tobacco users. Risk of CMM and MIS decreased with increasing smoking duration and quantity. Similarly, exclusive users of cigarettes, pipe and snuff were at reduced risks of CMM and MIS. Tobacco smoking was, however, unrelated to risk of CSCC. Snuff use was associated with a 40% decreased risk of CSCC, compared to non-tobacco users.

A BMI ≥25 kg/m^2 was associated with a 1.3-fold increased risk of CMM, compared to a BMI<25 kg/m^2, but there was no effect on risk of MIS, IMM or CSCC.

Azathioprine (Aza) treatment was found to considerably increase the risk of CSCC during all time periods analyzed post-transplantation. Additionally, a high accumulated dose of corticosteroids (Cs) after longer treatment durations conferred an increased risk of CSCC, compared to a very low accumulated dose of Cs. Cyclosporine treatment was unrelated to risk of CSCC. There were no significant associations between number or type of post-transplant infections and CSCC. HLA type and mismatch, number of transplantations and rejections, type of organ, as well as donor characteristics were not associated with risk of CSCC in OTRs.

Conclusions: In our study, tobacco use was associated with a decreased risk of CMM and MIS, but unrelated to risk of CSCC. Likewise, we found a relation between overweight/obesity and an increased risk of CMM, but not of CSCC, MIS or IMM. These findings need further research in order to understand the underlying mechanisms.

Post-transplant CSCC development seems not to be a result of immunosuppressive burden, instead we found important differences in risk conferred by specific immunosuppressive drugs. Other transplant-related factors were not associated with CSCC risk in our study. Future studies are required to further separate the direct carcinogenic, and the indirect immunosuppressive, effects of immunosuppressive drugs on risk of CSCC in OTRs and in other patient groups.

2 TABLE OF CONTENTS

3 LIST OF PUBLICATIONS

This thesis is based on the following four publications. They will be referred to in the text by their Roman numerals (I-IV).

I. Odenbro Å, Bellocco R, Boffetta P, Lindelöf B, Adami J.
Tobacco smoking, snuff dipping and the risk of cutaneous squamous cell carcinoma: a nationwide cohort study in Sweden.
Br J Cancer 2005; **92(7)**: 1326-8

II. Odenbro Å, Gillgren P, Bellocco R, Boffetta P, Håkansson N, Adami J.
The risk for cutaneous malignant melanoma, melanoma *in situ* and intraocular malignant melanoma in relation to tobacco use and body mass index.
Br J Dermatol 2007; **156**: 99-105

III. Odenbro Å, Smedy KE, Lindelöf B, Fernberg P, Bellocco R, Tufveson G, Höglund P, Adami J.
Immunosuppressive treatment after solid organ transplantation and risk of cutaneous squamous cell carcinoma.
Manuscript submitted.

IV. Odenbro Å, Smedy KE, Lindelöf B, Fernberg P, Bellocco R, Tufveson G, Höglund P, Adami J.
Infections and other risk factors for cutaneous squamous cell carcinoma in solid organ transplant recipients.
Manuscript.

4 LIST OF ABBREVIATIONS

ALG	Anti-lymphocyte Globulin (polyclonal antibody against lymphocytes)
ATG	Anti-thymocyte Globulin (polyclonal antibody against thymocytes)
Aza	Azathioprine (Imurel®, Imuran®)
BCC	Basal Cell Carcinoma
BMI	Body Mass Index
BSA	Body Surface Area
CI	Confidence Intervals
CMM	Cutaneous Malignant Melanoma
CMV	Cytomegalovirus
COX-2	Cyklooxygenase 2
Cs	Corticosteroids
CsA	Cyclosporine (Sandimmun®, Sandimmun Neoral®)
CSCC	Cutaneous Squamous Cell Carcinoma
EV	Epidermodysplasia Verruciformis
EBV	Epstein Barr Virus
GH	Growth Hormone
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HLA	Human Leukocyte Antigen
HSV	Herpes Simplex Virus
IARC	International Agency for Research on Cancer
IGF-1	Insulin-like Growth Factor 1
IMM	Intraocular Malignant Melanoma
IRR	Incidence Rate Ratio
KTR	Kidney Transplant Recipient
MC1R	Melanocortin-1 Receptor
MIS	Melanoma *in situ*
NMSC	Nonmelanoma Skin Cancer
OKT-3	Muromonab CD3 (Monoclonal antibody against CD3-receptor on T-cells)
OR	Odds Ratio
OTR	Organ Transplant Recipient
PAH	Polynuclear Aromatic Hydrocarbons
RR	Relative Risk or Rate Ratio
6-TG	6-Thioguanine
UVA	Ultraviolet Radiation A (wavelength 320-400 nm)
UVB	Ultraviolet Radiation B (wavelength 280-320 nm)
UVR	Ultraviolet Radiation
WHO	World Health Organization
VZV	Varicella Zoster Virus

5 INTRODUCTION

We are in the midst of an ongoing skin cancer epidemic. The incidence of skin cancer has increased more rapidly than any other cancer form in the fair-skinned population over the past several decades.[1, 2]

Prior to the industrial revolution, which reached Sweden around 1850, white, pale skin was considered an indicator of high social status, but this started changing early in the 20th century. Sunbathing behavior started with the introduction of "heliotherapy" - the treatment of diseases by sunlight exposure - by Rollier in 1927.[3] As a consequence, the possession of a tan came to be associated with good health and paleness with poor health. Coco Chanel, the famous fashion designer, re-enforced the popularity of sunbathing behavior by using the attribute of a suntan as a fashion statement.[3] In the beginning of the 1940s the first warnings emerged regarding a correlation between sunlight exposure and skin cancer.[3] These warnings continued and gained in credibility in the 1960s, 1970s, and 1980s, but still the sunbathing behavior has retained its popularity.

Figure 1. Newspaper feature from 1938 for sun protective milk spray. Cited text: "BATHERS at Willow Lake, near Glendale Calif., have adopted mass-production methods to speed up the process of acquiring coveted coats of sun tan. They employ a motor-driven atomizer to apply a newly developed milk spray, which is said to protect the skin from unaccustomed exposure to the sun's rays and to help prevent burning and peeling." Permission for reproduction from the owner of Modern Mechanix homepage.[4]

This behavior may be partially responsible for the increased incidence of skin cancer, although several observations indicate that other factors are likely to contribute as well. For instance, there are known differences in sex distribution and age distribution of cutaneous squamous cell carcinoma (CSCC) and cutaneous malignant melanoma (CMM) (see *Background*), albeit the mechanisms are yet unclear. Furthermore, the incidence of CMM is surprisingly high in Northern Europe, where the amount of ambient sunlight exposure reaching the earth is relatively low, compared to other parts of Europe.[5, 6] While the incidence of CMM has increased, the incidence of intraocular malignant melanoma (IMM) has rather decreased in the population since 1960.[7]

Additional to sunlight exposure, the population has been exposed to several other factors over the last century. In Sweden, the tobacco smoking habit started to spread from the end of the 19th century and steeply increased from after the Second World War to the 1970s, but has decreased since the 1980s.[8] Snuff use, on the other hand, dominated the Swedish tobacco market in the beginning of the 20th century. Thereafter, snuff use lost in popularity until the 1960s, but has since been increasing.[8] Furthermore, the proportion of overweight and obese individuals has increased rapidly during the last three decades in Sweden, and now includes more than 50% of men and almost 40% of women.[9, 10] Both tobacco use and overweight/obesity have been associated with an increased risk of many cancer types, but their relation to CSCC, CMM, and IMM are unclear. To shed further light on the etiology of these malignancies in relation to tobacco use and overweight/obesity, we designed two cohort studies originating from a large population-based cohort in Sweden.

The first successful kidney transplantation was performed between identical twins in Boston in 1954, but it was not until the introduction of azathioprine (Imurel®) as anti-rejection therapy in 1960 that this treatment of end-stage kidney disease became routine.[11] In Sweden, about 300-400 organ transplantations are performed every year and along with an increasingly effective immunosuppressive treatment, the 5-year survival rate has become excellent.[12] In 1968, the first report of an increased risk of malignancies in organ transplant recipients was published and this phenomenon is now well known.[13-15] The most common malignancy occurring post-transplantation is CSCC. To increase the understanding of the etiology of post-transplant CSCC, we conducted two case-control studies, nested in the Swedish population-based cohort of organ transplant recipients.

6 BACKGROUND

6.1 EPIDEMIOLOGY

Skin cancers are the most frequently occurring cancers in fair-skinned populations. The three main types of skin cancer are cutaneous squamous cell carcinoma (CSCC), basal cell carcinoma (BCC), and cutaneous malignant melanoma (CMM). CSCC and BCC are often referred to as nonmelanoma skin cancers (NMSC). The etiology and prognosis differ substantially between CSCC, BCC and CMM. This thesis will focus on CSCC and CMM as BCCs were not registered in the Swedish Cancer Register during the periods covered by the included studies (see *Subjects and methods*).

6.1.1 Cutaneous squamous cell carcinoma

CSCC is the malignant transformation of keratinocytes and is usually located on chronically sun-exposed skin, such as the face, hands, and underarms in males and females, and the scalp, neck, and ears specifically in males.[2, 16-18] Actinic keratosis is the principal precursor of CSCC and it is estimated that persons with multiple actinic keratoses have a cumulative life-time risk of an invasive CSCC of 6-10%.[2, 16, 17]

In Sweden, CSCC is the second most common cancer in males and the third most common cancer in females, constituting 9 and 7.5% of all cancers in men and women, respectively.[19] The risk of CSCC increases with age and the incidence rate of CSCC in men is about twice the incidence rate in women.[18, 19] From 1987 to 2006, the incidence rate doubled for both males and females, and was 62.6 per 100 000 in males and 32.0 per 100 000 in females in 2006 (**Figure 2**). CSCC has an invasive growth and may metastasize, but is usually detected and treated before this occurs. Therefore, mortality from CSCC is low in the general population. In organ transplant recipients (OTRs), however, the mortality is highly increased.[20]

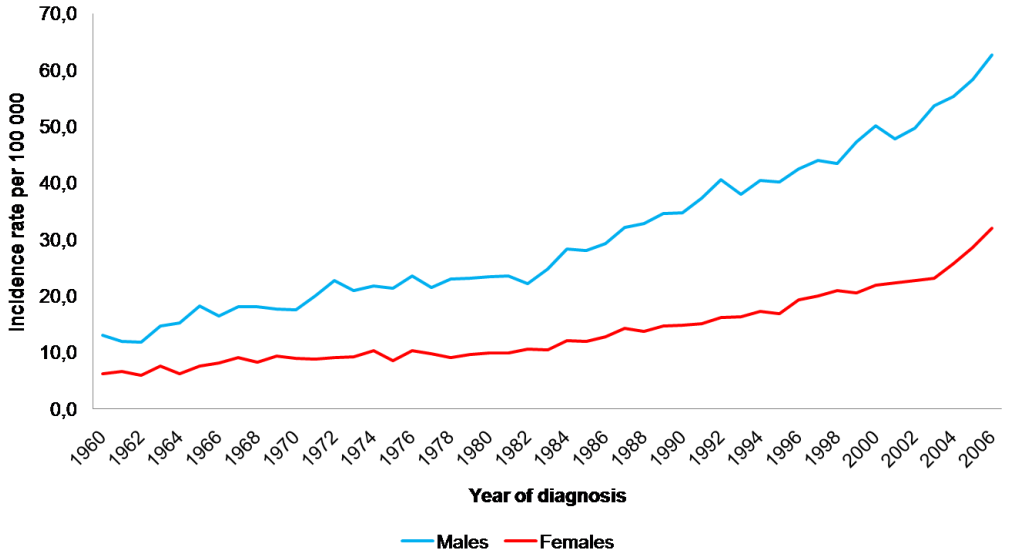

Figure 2. Trends in age-adjusted incidence rate (per 100 000) of cutaneous squamous cell carcinoma (CSCC) in males and females 1960-2006. Adapted from Cancer Incidence in Sweden 2006.[19]

6.1.2 Cutaneous malignant melanoma

CMM is the most malignant form of skin cancer. It originates from melanocytes and usually develop in the skin but may also arise in other locations such as the eye or in mucosal membranes (respiratory, digestive, and urogenital tract). CMM is categorized into four basic types according to the growth pattern, namely: superficial spreading melanoma (60-65% of all CMM), nodular melanoma (20-25%), lentigo malignant melanoma (5-7%) and acral lentiginous melanoma (5%).[21-23] The distribution of CMM differs from the distribution of CSCC with a higher proportion on the trunk in men and on the lower legs in women.[21, 23]

CMM has been one of the most rapidly increasing cancers in fair-skinned populations during the last decades.[1, 5, 22] In Sweden, the age-standardized incidence rate has doubled since 1980 (**Figure 3**) and CMM was in 2006 the seventh and sixth most common cancer in males and females, respectively.[19] Compared to other European countries, Sweden (and Norway) has a high incidence of CMM.[19, 21] CMM may occur at any age and is more common in females when diagnosed before 50 years of age and more common in males thereafter.[21] While the incidence rate has been increasing, the survival rate has improved. In 1960 the five-year survival rate was about 50% and in 2006 it had increased to about 90%.[1, 21] The improved survival is likely a consequence of earlier diagnosis since the median thickness of the tumor according to Breslow has decreased and a greater proportion of CMM are now diagnosed while *in situ* (>40% today compared to <10% in 1970).[1, 21]

Intraocular malignant melanoma (IMM) is a rare disease, representing only three percent of all malignant melanomas.[24] Still, IMM is the most frequent intraocular tumor and presents with a poor prognosis.[25] During the period 1960-1998 the age-standardized incidence rate of IMM in Sweden decreased from 11.7 to 8.4 per million in males and from 10.3 to 8.7 per million in females.[7] The same pattern has been observed in other countries.[26]

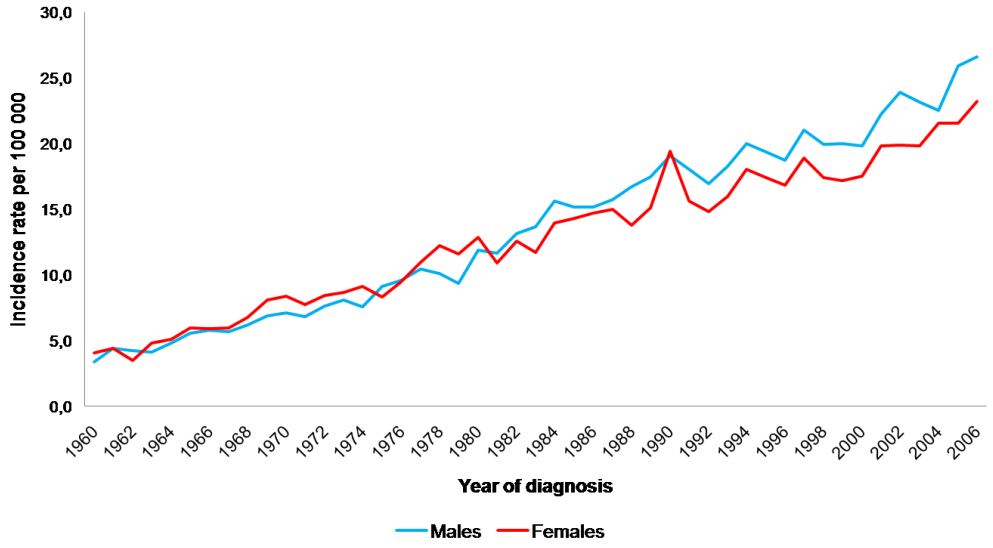

Figure 3. Trends in age-adjusted incidence rate (per 100 000) of cutaneous malignant melanoma (CMM) in males and females 1960-2006. Adapted from Cancer Incidence in Sweden 2006.[19]

6.2 RISK FACTORS

6.2.1 Tobacco smoking and snuff use

It is estimated that there are more than one billion smokers worldwide, and approximately three million deaths per year attributable to smoking.[27] This number is expected to increase, since smoking is gaining ground in developing countries.[27] Sweden has one of the lowest smoking prevalences in the world, with less than 20% daily smokers among men and women, and the prevalence has been decreasing since 1980 for men and since 1990 also for women.[28] In contrast, snuff use has increased in Sweden, particularly in men but also in women, and in 2004, 22 and 3% of men and women, respectively, were daily snuff users.[8, 29]

Tobacco smoking accounts for approximately 30% of all cancer cases in developed countries.[30] The International Agency for Research on Cancer (IARC) recently classified tobacco smoking as the cause of cancer at more organ sites than any other human carcinogen.[30] Tobacco smoke contains about 4 000 constituents whereof 69 are classified as carcinogens, including polynuclear aromatic hydrocarbons (PAHs), aromatic amines, *N*-nitrosamines, aldehydes, miscellaneous organic compounds, and inorganic compounds.[30] These compounds damage DNA in a number of ways by, for example, inducing sister chromatid exchanges, DNA strand breaks, micronuclei, DNA adducts, and oxidative damage.[27, 31, 32] Tobacco smoking also affect the immune system at several levels. This is demonstrated by the increased risk of infections in smokers and the decreased risk of some autoimmune and inflammatory diseases.[33, 34] However, the effect on the immune system is complex. For one, the white cell blood count is increased in smokers compared to non-smokers and the CD4+ to CD8+ T-cell ratio may be modified.[35-38] Some studies suggest a T-cell anergy whereas others do not.[35, 39] Furthermore, decreased serum concentration of immunoglobulins and lysozyme,[36] decreased natural killer (NK) cell activity,[40] and decreased secretion of pro-inflammatory cytokines (IL-1, IL-6, TNF-α) from macrophages have been reported in smokers compared to non-smokers.[35] Many constituents of tobacco smoke may exert these effects, e.g. PAH and nicotine have been shown to be involved, but the immunosuppressive effect of tobacco smoking is probably also a consequence of the stimulation of catecholamine- and ACTH-release that inhibits immune responses.[35, 41]

Tobacco smoking is strongly related to cancer at many sites, for example cancer of the lung, bladder, larynx, uterine cervix, etc.[42] The skin is affected by smoking in several ways, including impaired wound healing, increased wrinkling of the face, premature skin aging, and decreased skin blood flow.[41, 43-46] The evidence of an association between smoking and CSCC is however scarce (**Table 1**). Grodstein *et al.*[47] reported on the association from a large prospective cohort study in the USA in 1995. They found an increased risk of CSCC in current cigarette smokers (relative risk (RR) 1.5, 95% confidence interval (CI), 1.1-2.1) compared to non-smokers. The risk was not increased in former cigarette smokers, and there was no trend with increasing number of cigarettes smoked per day. In a hospital-based case-control study from the Netherlands in 2001,[48] current smokers of any tobacco smoking product were reported to have a 2.9-fold (95% CI, 1.5-5.6) increased risk of CSCC, and former smokers a 1.8-fold (95% CI, 1.0-3.0) increased risk, compared to non-smokers. The risk also increased with increasing numbers of cigarettes smoked per day

(p_{trend}=0.004), and trend in risk of CSCC by duration of smoking was borderline significant (p_{trend}=0.08). In a smaller case-control study from Canada,[49] ever cigarette smokers were found to have a 2.3-fold increased risk of CSCC (95% CI, 1.3-4.2), compared to never-smokers, no dose-response relationship was found for number of cigarettes smoked per day in this study. In contrast, no association could be detected between smoking and CSCC in two case-control studies, and in one cohort study.[50-52]

Table 1. Summary of previous studies investigating the association between tobacco smoking and risk of cutaneous squamous cell carcinoma (CSCC).

Author, year	**Study design**	**No. of cases/ controls or cohort**	**RR/OR (95% CI), ever use**†	**RR/OR (95% CI), dose/duration**†
Odenbro *et al.* 2005[53]	Cohort, retrospective ♂	756/ 337 311	Former smoker: **1.0** (0.8-1.2) Current smoker: **1.0** (0.8-1.2) Ever cigarette: **1.0** (0.9-1.3) Ever pipe: **0.8** (0.6-1.0) Ever cigar: **0.9** (0.4-1.7) Ever snuff: **0.6** (0.4-0.9)	No. of cigarettes/day: 1-10: **1.1** (0.9-1.4) 11-20: **0.9** (0.7-1.2) ≥21: **0.9** (0.6-1.4)
Efird *et al.* 2002[52]	Cohort, retrospective ♂+♀	822/ 3 662 (USA)	Smoking is not associated with risk of CSCC. No estimates given.	
Grodstein *et al.* 1995[47]	Cohort, prospective ♀	191/ 121 700 (USA)	Former cigarette smoker: **1.1** (0.8-1.5)* Current cigarette smoker: **1.5** (1.1-2.1)*	No. of cigarettes/day (current smokers): 1-14: **1.3** (0.7-2.2)* 15-24: **1.8** (1.2-2.8)* ≥25: **1.3** (0.7-2.3)*
De Hertog *et al.* 2001[48]	Case-control ♂+♀	161/ 386 (Netherlands)	Former cigarette smoker: **1.8** (1.0-3.0)* Current cigarette smoker: **2.9** (1.5-5.6)* Ever pipe: **6.7** (1.9-23.8)* Ever cigar: **1.6** (0.6-4.2)*	No. of cigarettes/day (current smokers): <11: **2.4** (1.0-6.0)* 11-20: **3.0** (1.3-7.1)* ≥20: **4.1** (1.5-11.5)*
Kune *et al.* 1992[50]	Case-control ♂+♀	88/ 88 (Australia)	Smoking is not associated with risk of CSCC. No estimates given.	
Hogan *et al.* 1990[51]	Case-control ♂+♀	178/ 284 (Canada)	Smoking is not associated with risk of CSCC. No estimates given.	
Aubry *et al.* 1984[49]	Case-control ♂+♀	92/ 174 (Canada)	Ever-smoker: **2.3** (1.3-4.2)*	No dose-response relationship. No estimates given.
Risk of subsequent CSCC in patients with prior CSCC or BCC				
Karagas *et al.* 1992[54]	Cohort, prospective ♂+♀	132/ 1805 (USA)	Former cigarette smoker: **1.6** (1.1-2.5)*	No. of cigarettes/day (current smokers): 1-20: **1.9** (1.0-3.4)* 21-40: **2.1** (1.0-4.3)* ≥41: **3.3** (1.1-9.6)*

*Results adjusted for sun exposure and/or constitutional characteristics
†Compared to never smokers

Pipe and cigar smoking in relation to risk of CSCC has been investigated previously in one case-control study.[48] Cigar smokers were associated with a non-significant 1.6-fold increased risk of CSCC, while pipe smokers had a substantially increased risk of CSCC (odds ratio (OR), 6.7; 95% CI, 1.9-23.8), but these estimates were based on

few cases. The relation between smoking and CSCC has also been investigated in other patient groups. Karagas *et al*., 1992,[54] investigated the risk of a subsequent CSCC in patients with prior skin cancer and found an increased risk of CSCC in smokers in this patient group (RR 1.6; 95% CI, 1.1-2.5), compared to non-smokers. In organ transplant recipients, some studies have found an increased risk of CSCC in current[55] and former[55, 56] smokers but the majority have not found an association[57-60]. However, these studies were generally impeded by small size.[55, 57, 59, 60]

The composition of smokeless tobacco varies in different parts of the world. Swedish oral moist snuff has comparatively low levels of some harmful substances, such as tobacco-specific nitrosamines, compared to other types of smokeless tobacco. Most studies on the association between Swedish snuff use and cancer risk (lung and head/neck) have not found any associations,[61-63] although there are some previous studies that have presented evidence of an association with cancer of the pancreas[61] and esophagus[64]. No studies investigating the association between snuff use and risk of CSCC were found.

The association between smoking and CMM has been somewhat more thoroughly examined (**Table 2**) than the association with CSCC.

Table 2. Summary of previous studies investigating the association between tobacco smoking and snuff use and risk of cutaneous malignant melanoma (CMM).

Author, year	**Study design**	**No. of cases/ controls or cohort**	**RR/OR (95% CI), ever, former, current use**[†]	**RR/OR (95% CI), dose/duration**[†]
Odenbro *et al.* 2007[65]	Cohort, retrospective ♂	1309/ 339 802 (Sweden)	Former smoker: **0.8** (0.6-0.9) Current smoker: **0.7** (0.6-0.8) Ever cigarette: **0.7** (0.6-0.8) Ever pipe: **0.6** (0.5-0.8) Ever cigar: **1.0** (0.6-1.7) Ever snuff: **0.6** (0.5-0.8)	Cigarette-years (amount of tobacco smoked/day X duration): 1-499: **0.8** (0.7-1.0) 500-999: **0.6** (0.5-0.7) ≥1000: **0.4** (0.3-0.6)
Freedman *et al*. 2003[66]	Cohort, prospective ♂/♀	207/ 68 588 (USA)	Former smoker: ♂: **0.6** (0.3-1.2)* ♀: **1.1** (0.7-1.5)* Current smoker: ♂: **0.6** (0.3-1.3)* ♀: **0.8** (0.5-1.3)*	Pack-years: <10, ♂: **0.7** (0.3-1.6)* 10-29, ♂: **0.6** (0.2-1.3)* ≥30, ♂: **0.5** (0.2-1.3)* <10, ♀: **1.0** (0.7-1.5)* 10-29, ♀: **0.8** (0.5-1.2)* ≥30, ♀: **0.8** (0.4-1.7)*
Veierød *et al.* 1997[67]	Cohort, prospective ♂+♀	108 / 50 757 (Norway)	Former smoker: **0.9** (0.5-1.4)	No. of cigarettes/day: <11: **0.6** (0.4-1.1) ≥11: **0.7** (0.4-1.4)
Le Marchand *et al*. 2006[68]	Case-control, population-based ♂/♀	278 (167)/ 167 (Hawaii, USA)	Former smoker: ♂: **0.9** (0.5-1.4) ♀: **0.9** (0.5-1.8) Current smoker: ♂: **0.5** (0.3-1.0) ♀: **0.5** (0.2-1.2)	
De Hertog *et al.* 2001[48]	Case-control ♂+♀	125/ 386 (Netherlands)	Ever cigarette smoker: **0.8** (0.5-1.3)*	
Shors *et al.*	Case-control	386/ 727	Former smoker:	

2001[69]	♂+♀	(USA)	**0.8** (0.6-1.1) Current smoker: **0.6** (0.4-0.8)	
Green *et al.* 1999[70]	Case-control ♂+♀	275/ 496 (Australia) 36/72 (Scotland)	Former smoker: **0.8** (0.6-1.1) Current smoker: **0.6** (0.4-0.9)	
Westerdahl *et al.* 1996[71]	Case-control, population-based ♂+♀	400/ 640 (Sweden)	Former cigarette smoker: **1.0** (0.3-3.5)* Current cigarette smoker: **0.7** (0.5-1.1)* Ever pipe/cigar smoker: **0.9** (0.5-1.7)*	No. of cigarettes/day: 1-19: **0.7** (0.5-1.1) ≥20: **0.6** (0.3-1.1)
Siemiatycki *et al.* 1995[72]	Case-control ♂	103/ 533 (1602 cancer controls) (Canada)	Ever smoker: **0.5** (0.3-0.9)	Cigarette-years: <500: **0.7** (0.4-1.4) 501-1000: **0.5** (0.2-0.9) 1001-1500: **0.4** (0.2-1.0) ≥1501: **0.4** (0.1-0.9)
Østerlind *et al.* 1988[73]	Case-control, population-based ♂+♀	474/ 926 (Denmark)	Former smoker: **1.0** (0.7-1.3)º Current cigarette smoker: **0.8** (0.6-1.0)º Current pipe: **1.2** (0.7-2.3)º Current cigar/cigarillo: **1.1** (0.7-1.7)º	No. of cigarettes/day: <10: **1.1** (0.7-1.5)º 10-19: **0.6** (0.4-0.9)º ≥20: **0.8** (0.6-1.2)º
Green *et al.* 1986[74]	Case-control ♂/♀	183/ 236 (Australia)	Ever smoker: ♂: **1.0**§ ♀: **0.5**§	Life-time no. of packs, ♂+♀: 1-499: **0.7** (0.2-2.1) 500-9999: **0.8** (0.3-2.4) ≥10000: **1.3** (0.3-5.0)
Gallagher *et al.* 1986 [75]	Case-control ♂+♀	595/ ? (Canada)	No significant association with cigarette, pipe and cigar smoking, or to pack-years. No estimates given.	
Williams *et al.* 1977[76]	Case-control (cancer-controls) ♂/♀	?/ ? (7 518 in total study group) (USA)		Cigarette-years: 1-400, ♂: **1.1**§ 401-800, ♂: **0.4**§ ≥801, ♂: **0.5**§ 1-400, ♀: **1.1**§ 401-800, ♀: **0.4**§ ≥801, ♀: **0.9**§ Cigar-years: 1-50, ♂: **2.4**§, ≥50: **0.5**§ Pipe-years: Low, ♂: **0.9**§, High: **1.4**§ Chewing or snuff tobacco-years: Low, ♂: **0.6**§, High: -

*Results adjusted for sunlight exposure and/or constitutional characteristics
†Compared to never smokers
ºResults not adjusted for sunlight exposure and constitutional characteristics, but the authors state in the text that the estimates did not change upon adjustment.
§Significance of association not given. Crude OR.

Neither of two large prospective cohort studies investigating risk factors for CMM found a significant association with smoking.[66, 67] However, in both these studies there was an indicated decreased risk of CMM with increasing dose[67], increasing duration[66] (p_{trend}=0.03), and increasing number of pack-years[66] (p_{trend}=0.03) of smoking compared to never smoking. A similar trend of reduced risk with increasing dose or duration of smoking has been observed in several previous case-control studies. Westerdahl *et al*.,

1996,[71] reported a 30% reduced risk of CMM in current smokers (95% CI, 0.5-1.1), compared to non-smokers, and this risk decreased in individuals who smoked 20 or more cigarettes per day (OR 0.6; 95% CI, 0.3-1.1). In a somewhat smaller case-control study, male ever cigarette smokers had a 0.5-fold decreased risk of CMM (95% CI, 0.3-0.9) compared to never smokers.[72] Again, this risk was reduced with increasing cigarette-years (**Table 2**).[72] Shors *et al*., 2001,[69] and Green *et al*., 1999,[70] both found a statistically significant 40% decreased risk of CMM and CMM of the soles and palms, respectively, in current smokers compared to never smokers. Less prominently, and without a dose-response relationship, two other case-control studies found a somewhat reduced risk of CMM in current cigarette smokers compared to never smokers.[73, 74] Williams *et al*., 1977,[76] observed a decreased risk of CMM with increasing cigarette-years but this effect disappeared when they excluded cancer-controls with a cancer known to be associated with smoking. Lastly, cigarette smoking was found to be unassociated with risk of CMM in two other case-control studies.[48, 75] Current pipe and cigar smoking was investigated in three previous studies and were found to be unrelated to risk of CMM.[71, 73, 75] Another study also investigated the role of cigar and pipe smoking as well as snuff use, but the estimates were based on very few cases and were therefore difficult to interpret.[76]

Smoking in relation to IMM appears to only have been investigated in one prior study. This case-control study found no association between smoking and risk of IMM.[77]

6.2.2 Body mass index

Body mass index is a measure of weight in relation to height and is calculated as weight (kg) divided by height (m) squared (unit: kg/m^2). The World Health Organization (WHO) has classified BMI in five categories, namely underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5-24.9 kg/m^2), grade 1 overweight ("overweight", BMI 25-29.9 kg/m^2), grade 2 overweight ("obesity", BMI 30-39.9 kg/m^2), and grade 3 overweight ("morbid obesity", BMI ≥40 kg/m^2). The prevalence of overweight and obese individuals has increased markedly during the last two decades, foremost in industrialized countries, but also in many developing countries.[78, 79] By the end of year 2000, nearly two thirds of adults in the United States were overweight or obese, and there were 300 million obese adults worldwide.[78] In Sweden, the prevalence of overweight and obesity is not as extreme as in the United States, but it has grown with increasing pace during the same period (**Figure 4**).[9]

It is well known that obesity is related to risk of diabetes (type II) and cardiovascular diseases. However, the association between obesity and different types of cancer has received less attention. Epidemiological studies have indicated that obesity contributes to the increased incidence of many cancers, for example of the colon, breast (in premenopausal women), endometrium, kidney (renal cell), oesophagus (adenocarcinoma), gastric cardia, pancreas, and gall bladder.[78] There are a number of proposed mechanisms by which obesity might contribute to tumor formation, including chronic hyperinsulinemia, increased concentrations of growth hormone (GH) and insulin-like growth factor-1 (IGF-1), altered concentrations of endogenous sex steroids, and maintenance of a chronic inflammation.[78, 80]

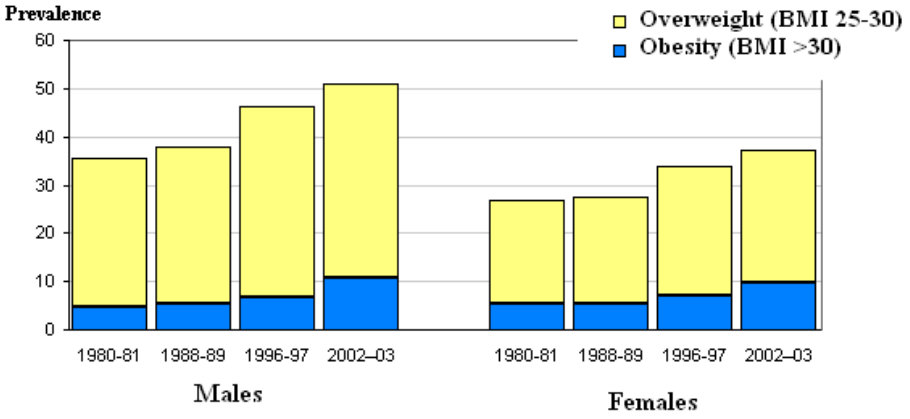

Figure 4. Prevalence of overweight (BMI 25-30 kg/m^2) and obesity (BMI >30 kg/m^2) in Sweden in 16-84 year old males and females during different time periods. Adapted from Statistics Sweden.[10]

Only one study has studied the association between overweight/obesity and risk of CSCC. This population-based retrospective cohort study, including 28 129 patients with a discharge diagnosis of obesity during 1965-1993 in Sweden, did not find a relation between obesity and risk of CSCC.[81] Two other studies, one investigating the risk of a subsequent cancer after a CSCC diagnoses[52] and one investigating Arsenic methylation in relation to risk of CSCC,[82] have reported a lower BMI in CSCC patients compared to comparison subjects, but these studies did not present adjusted estimates.

Table 3 displays studies investigating BMI in relation to CMM occurrence. This table is not complete, one study conducted in the same cohort as Study II,[83] one study with a low number of cases,[81] and one study not giving point estimates[84] are not displayed in the table. Although the minority is significant[65, 83, 85, 86] and few have found a dose-response relationship[83, 86], a high BMI has often been associated with an increased risk of CMM in males, compared to a low BMI.[66, 69, 87] In one cohort study, based on few cases, there was no association between BMI and CMM in males,[81] and another case-control study indicated a non-significant decreased risk of CMM with increasing BMI.[68] Increasing height[67-69, 85] and weight[69, 86] has been inconsistently associated with an increased risk of CMM in males. An increasing body surface area (BSA)[67, 69, 85, 86, 88] has more consistently been associated with a higher risk of CMM.

Olsen *et al*., 2008,[89] performed a pooled analyses of anthropometric measures in relation to risk of CMM in women. Using data from eight case-control studies, they found that women in the highest quartile of height had an increased risk of CMM (OR 1.3; 95% CI, 1.1-1.6), compared to the lowest quartile of height. No effect by BSA, weight or BMI was observed. These results are in concordance with two larger and one smaller prospective cohort study with regard to BMI.[66, 85, 90] Thune *et al*., 1993,[85] found an increased risk of CMM in women with increasing height and BSA in a large prospective cohort study. BSA was also associated with risk of CMM in a prospective cohort study conducted by Veierød *et al*., 2003,[91] whereas neither height nor weight was associated with risk of CMM in women in another prospective cohort study.[66]

No studies were found investigating the relation between BMI and IMM.

Table 3. Summary of previous studies investigating the association between body mass index (BMI) and risk of cutaneous malignant melanoma (CMM).

Author, year	Study design	No. of cases/ controls or cohort	RR/OR (95% CI) ♂	RR/OR (95% CI) ♀
Odenbro *et al*. 2007[65]	Cohort, retrospective ♂	1 309/ 339 802 (Sweden)	*BMI according to WHO:* <25: ref ≥25: **1.3** (1.2-1.5)	
Lukanova *et al*. 2006[87]	Cohort, prospective ♂/♀	44 ♂/ 33 424 48 ♀/ 35 362 (Sweden)	*BMI in quartiles:* 18.5-23.4: ref 23.5-25.3: **1.7** (0.7-5.0) 25.4-27.6: **1.9** (0.8-5.5) ≥27.7: **2.0** (0.8-5.8)	*BMI in quartiles:* 18.5-22.1: ref 22.2-24.2: **2.6** (1.1-7.1) 24.3-27.1: **1.4** (0.5-4.2) ≥27.2: **2.6** (1.0-7.2)
Freedman *et al*. 2003[66]	Cohort, prospective ♂/♀	243/ 68 588 (USA)	*BMI in quartiles:* <23.4: ref 23.4-25.1: **2.7** (1.1-7.0)* 25.2-27.4: **2.1** (0.8-5.6)* ≥27.5: **1.4** (0.5-4.1)*	*BMI in quartiles:* <20.4: ref 20.4-22.1: **0.8** (0.5-1.3)* 22.2-24.7: **0.9** (0.6-1.4)* ≥24.8: **0.9** (0.6-1.4)*
Thune *et al*. 1993[85]	Cohort, prospective ♂/♀	2 144 ♂ / 659 689 2 814 ♀/ 736 876 (Norway)	*BMI in quintiles:* I: ref II: **1.2** (1.0-1.3)¶ III: **1.3** (1.1-1.5)¶ VI: **1.3** (1.2-1.5)¶ V: **1.3** (1.1-1.5)¶	*BMI in quintiles:* I: ref II: **1.1** (1.0-1.2)¶ III: **1.1** (1.0-1.2)¶ IV: **1.0** (0.9-1.1)¶ V: **0.9** (0.8-1.0)¶
Gallus *et al*. 2006[86]	Case-control, hospital-based ♂/♀	542/ 538 (Italy)	*BMI in quartiles:* I: ref II: **1.5** (0.8-2.7)* III: **2.1** (1.1-4.0)* IV: **1.9** (1.0-3.5)*	*BMI in quartiles:* I: ref II: **1.3** (0.8-2.2)* III: **1.2** (0.7-2.1)* IV: **2.1** (1.2-3.6)*
Le Marchand *et al*. 2006[68]	Case-control, population-based ♂/♀	278 (167)/ 167 (Hawaii, USA)	*BMI in quartiles:* <23.9: ref 23.9-26: **0.6** (0.3-1.2) 26.1-28.6: **0.6** (0.3-1.2) ≥28.7: **0.7** (0.4-1.2)	*BMI in quartiles:* <20.5: ref 20.5-22.8: **0.7** (0.3-1.4) 22.9-26.1: **0.8** (0.4-1.8) ≥26.2: **0.5** (0.2-1.0)
Shors *et al.* 2001[69]	Case-control ♂/♀	386/ 727 (USA)	*BMI in quartiles:* <24.4: ref 24.5-26.4: **1.5** (0.8-2.8)* 26.5-26.6: **1.4** (0.7-2.6)* ≥26.7: **1.6** (0.9-2.9)*	*BMI in quartiles:* <22.0: ref 22-24.7: **1.2** (0.7-2.1)* 24.8-28.0: **1.1** (0.6-1.9)* ≥28.1: **1.0** (0.6-1.7)*
Reeves *et al.* 2007[90]	Cohort, prospective ♀	1 635/ 1 222 630 (UK)		*BMI according to WHO:* 22.5-24.9: ref 25.-27.4: **1.1** (1.0-1.2) 27.5-29.5: **0.9** (0.8-1.1) ≥30: **0.9** (0.8-1.1)
Smith *et al*. 1998[92]	Case-control, ♀	308/ 233 (USA)		*BMI after nomogram:* ≤23: ref 23.1-28.5: **1.3** (0.8-2.1)* ≥28.6: **1.2** (0.7-2.2)*
Naldi *et al*. 2005[88]	Case-control, hospital-based ♀	316/ 308 (Italy)		*BMI in tertiles:* <23: ref 23-26.9: **1.2** (0.8-1.8)* ≥27: **2.0** (1.2-3.2)*
Veierød *et al*. 1997[67]	Cohort, prospective ♂+♀	108/ 50 757 (Norway)	*BMI in quartiles, ♂+♀:* <22.6: ref 22.6-24.5: **1.1** (0.7-1.9) § 24.6-26.8: **1.1** (0.6-1.9) § ≥26.9 : **0.9** (0.9-1.5) §	
Kirkpatrick *et al*. 1994[93]	Case-control ♂+♀	234/ 248 (USA)	*BMI in quartiles, ♂+♀:* I: ref II: **1.5** (0.9-2.4) III: **1.7** (1.0-2.8) IV: **1.9** (1.1-3.3)	

*Results adjusted for sunlight exposure and/or constitutional characteristics.
¶ Results adjusted for birth cohort and geographic region as proxy for sun exposure.
§ Results adjusted for geographic region.

6.2.3 Immunosuppression after organ transplantation

The first kidney transplantation was performed in 1964 in Sweden, but kidney and, later, other solid organ transplantations did not become routine procedure until the 1970s (**Figure 5**). The number of transplantations performed per year gradually increased from 1970 to 1997 but has been stable thereafter.

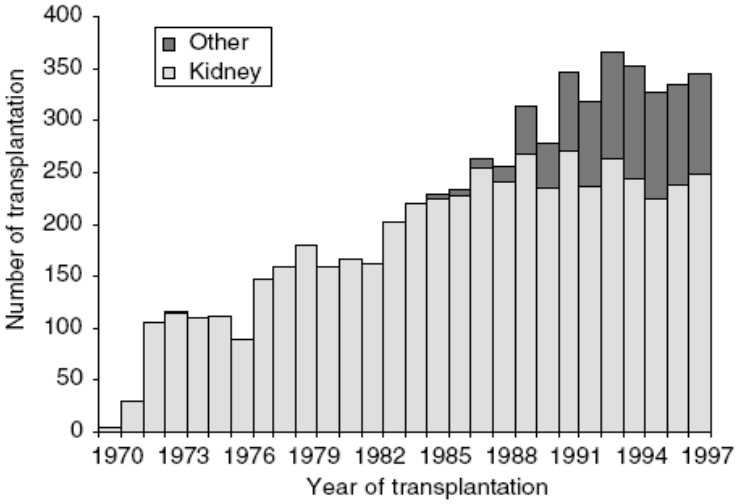

Figure 5. Total number of patients undergoing kidney (light grey bars) and other organ (dark grey bars) transplantation in Sweden per year 1970-1997. Adapted from Adami *et al.* 2003.[94]

It is well established that OTRs have an increased risk of malignancies.[13-15, 94] CSCC is the most common malignancy occurring post-transplantation and accounts for approximately 50% of all cancers arising *de novo*.[94, 95] OTRs have up to a 250-fold increased risk of CSCC,[94, 96-98] and the CSCC to BCC ratio is reversed (>1), compared to the general population.[98, 99] Similar to the general population, sunlight exposure, latitude of residence, and constitutional characteristics are associated with risk of CSCC post-transplantation.[56, 59] CSCC incidence also increases with time after transplantation - at 10 years post-transplantation cumulative incidences of about 10 and 50% have been reported in Europe and Australia, respectively, and at 20 years post-transplantation these incidences increased to about 20-40 and 70-80%.[56, 94, 97, 100] With increasing age at transplantation, CSCC occurs sooner and with an increased frequency.[98, 99] However, after a lag time of 5-10 years, the risk of CSCC in comparison to the general population is higher in younger OTRs than in older OTRs.[94, 98]

OTRs receive lifelong pharmacological immunosuppressive therapy to prevent rejection of the organ. Immunosuppressive medication consists of corticosteroids (Cs: prednisolone, methylprednisolone, hydrocortisone, betamethasone, and cortisone), antiproliferative agents (azathioprine (Aza) and mycophenolate mofetil), calcineurin inhibitors (cyclosporine (CsA), cyclosporine microemulsion, and tacrolimus), mTOR inhibitors (sirolimus and everolimus), and antibody preparations directed at lymphocytes or cytokines (such as anti-thymocyte globulin (ATG), anti-lymphocyte globulin (ALG), CD-3 muromonab (OKT-3), daclizumab, etc.). In the early times of organ transplantation, Aza (Imurel®) and corticosteroids (usually Medrone®,

Deltison® or Prednisolone®) were the only available immunosuppressive agents and were given to all OTRs. From the beginning of the 1980s, CsA (Sandimmun®) was gradually introduced in Sweden, either together with Cs or, more commonly, in triple combination therapy with Aza and Cs. Starting in the early 1990s, cyclosporine microemulsion (Sandimmun Neoral®) gradually replaced CsA, and from the late 1990s, mycophenolat mofetil (CellCept®) was introduced as an alternative to Aza and tacrolimus (Prograf®) as an alternative to CsA. Sirolimus (Rapamune®) was not used until after year 2000. All these immunosuppressive agents target the immune system through different mechanisms, and since the studies in this thesis investigate the role of Aza, CsA and Cs, my focus will be on these drugs.

Aza is a prodrug that is converted to 6-mercaptopurine in the body. 6-mercaptopurine is thereafter converted through thio-inosine-monophosphate to 6-thioguanine (6-TG). 6-TG is a purine analogue that is incorporated in the DNA, inhibiting its synthesis and the proliferation of T- and B-lymphocytes.[101, 102] Aza is an unspecific drug not only acting on immune cells, but on all replicating cells, and it has been shown that Aza is mutagenic.[103] CsA inhibits calcineurin and thereby prevents activation of NF-AT induced transcription of cytokine genes, such as IL-2.[101, 102] Consequently, IL-2 promoted T- and B-cell proliferation is inhibited.[102] Apart from CsA´s effect on the immune system, Hojo *et al*., 1999,[104] demonstrated with experiments on cells *in vitro* and in animals *in vivo*, that CsA may promote cancer progression by a direct cellular effect. Lastly, Cs diffuses freely across cell membranes, binds to cytoplasmic receptors, and translocates to the nucleus where it affects the transcription of genes involved in the immune response.[101] By this route, CS influences the number, distribution and function of all leukocytes.[101]

Although the increased risk of CSCC in OTRs has largely been attributed to the immunosuppressive treatment,[105, 106] the underlying mechanisms are unclear. Several studies have crudely investigated the risk of CSCC in relation to treatment regimens,[56, 95, 96, 100, 107-112] but studies with assessment of treatment dose and duration are rare[55, 113-117] (**Table 4**). Moreover, some studies did not clearly separate drugs included in different treatment groups.[95, 108, 110] With some exceptions,[56, 95, 96, 100, 107] these studies were also impeded by limited statistical power to detect a difference between treatment groups, and sometimes grouped different skin malignancies[95, 107, 108, 112] in the analyses. Nevertheless, the majority of prior studies have not found a difference in risk of CSCC with different treatment regimens.[55, 95, 100, 107, 109, 111, 112] Some studies have found an increased risk with triple treatment (Aza+CsA+Cs) compared to double treatment with Aza+Cs.[96, 108, 110] Based on these findings it has been hypothesized that the risk of CSCC post-transplantation is primarily mediated by a total immunosuppressive drug load rather than by specific drugs. However, in a retrospective cohort study including 361 kidney transplant recipients (KTR), Ramsay *et al*., 2003,[56] found that ever treatment with Aza increased the risk of CSCC 2.4-fold (p=0.02). Aza treatment has also been observed to increase risk of CSCC in three other studies,[108, 113, 114] of which one prospective cohort study[113] and one randomized clinical trial[114] had assessed

Table 4. Summary of previous studies investigating the association between immunosuppressive drugs and risk of cutaneous squamous cell carcinoma (CSCC) or nonmelanoma skin cancer (NMSC).

Author, year	Study design	No of NMSC / No. OTR*	Findings or RR/OR (95% CI)**
Dantal *et al.* 1998[114]	Randomized clinical trial (France)	23 **CSCC**/ 231 KTR	A higher frequency of NMSC occurred in the high CsA dose group. This group also received higher mean doses of Aza.
Fortina *et al.* 2004[115]	Cohort, prospective (Italy)	26 **CSCC**/ 230 HTR	Cumulative doses/kg of Aza, CsA, Cs separately were not associated with risk of CSCC but a weighted linear combination of Aza+CsA+Cs was associated with an increased risk of CSCC after ≥3 years of follow-up.
Ramsay *et al.* 2003[56]	Cohort, retrospective (Australia)	135 **CSCC**/ 361 KTR	Aza+CsA+Cs: ref Aza+CsA: **0.5** (0.2-1.4) Aza+Cs: **0.8** (0.3-2.1) CsA+Cs: **0.4** (0.2-1.0)
Fuente *et al.* 2003[112]	Cohort, prospective (Spain)	39 **NMSC**/ 174 KTR	CsA+Cs: ref Aza+CsA+Cs: **0.7** (0.3-1.4)
Naldi *et al.* 2000[109]	Cohort, retrospective (Italy)	33 **CSCC**/ 1 062 KTR, 267 HTR	Aza+Cs: ref CsA+Cs: **1.0** (0.3-2.8) Aza+CsA+Cs: **1.3** (0.5-3.3)
Jensen *et al.* 1999[96]	Cohort, retrospective (Norway)	97 **CSCC**/ 2 397 KTR+ 164 HTR	Aza+Cs: ref CsA+Cs: **1.3** (0.4-3.8) Aza+CsA+Cs: **2.8** (1.4-5.3)
Hiesse *et al.* 1997[108]	Cohort, retrospective (France)	52 **NMSC**/ 1 710 KTR	ISR without CsA: **0.6** (p=0.02) ISR without Aza: **0.2** (p=0.03)
Bouwes Bavinck *et al.* 1996[100]	Cohort, retrospective (Australia)	148 **CSCC**/ 1 098 KTR	CsA: ref Aza <1980: **1.3** (0.7-2.3) Aza ≥1980: **1.1** (0.6-2.0) Aza+CsA: **1.1** (0.6-1.8)
Montagnino *et al.*1996[111]	Cohort, retrospective (Italy)	20 **CSCC**/ 737 KTR	Aza+Cs: ref CsA: **0.4** (p=0.38) CsA+Cs: **0.5** (p=0.34) Aza+CsA+Cs: **0.4** (0.24)
Sheil *et al.* 1991[107]	Cohort, retrospective (Australia)	1035 **NMSC**/ 5 897 KTR	There was no difference in skin cancer occurrence between Aza- and CsA-treated patients.
Gruber *et al.* 1994[95]	Cohort, retrospective (USA)	90 **NMSC**/ 1 887 KTR	Aza+Cs+ALG: ref Aza+CsA+Cs+ALG/ Aza+CsA+Cs/CsA+Cs: p=0.97
Kinlen *et al.* 1979[113]	Cohort, prospective (UK and Australia)	62 **CSCC**/ 3 823 KTR+ 1 349 immunosuppressed non-transplanted	Patients that developed skin cancer received a higher mean dose of Aza during the first six months.
Odenbro *et al.* 2008[117]	Case-control, (Sweden)	207 **CSCC**/ 189 OTR	CsA+Cs: ref Aza+Cs: **4.6** (1.5-13.8) Aza+CsA+Cs: **5.7** (2.0-15.8)
Bouwes-Bavinck *et al.* 1991[116]	Case-control. (Netherlands)	30 **CSCC**/ 131 KTR	Cumulative dose of Aza was not associated with risk of CSCC. There was an indicated reduced risk of CSCC with increasing dose of CS.
Ramsay *et al.* 2000[55]	Cross-sectional / longitudinal (UK)	28 **CSCC**/ 182 KTR	ISR, mean dose/cumulative dose, and dose per body weight (kg) were not associated with risk of NMSC.
Glover *et al.* 1997[110]	Cross-sectional study (UK)	21 **CSCC**/ 265 KTR	Aza+Cs: ref Aza+CsA+Cs: **8.4** (1.3-54.8)

* OTR= organ transplant recipients, KTR= kidney transplant recipients, HTR= heart transplant recipients,
** ISR= immunosuppressive regimen, Aza= azathioprine, CsA= cyclosporine, Cs= corticosteroids

doses of Aza administered. In two of these studies, CsA was also found to be associated with risk of CSCC.[108, 114] Cumulative dose of Aza was not associated with risk of CSCC in three small studies.[55, 115, 116]

The association between Cs and risk of CSCC in OTRs has only been investigated in three small studies previously, all of which found CSCC to be unrelated with increasing cumulative dose of Cs.[55, 115, 116] In the non-transplant population, the role of Cs in CSCC occurrence has been investigated in one large retrospective cohort study[118] and one large case-control study[119]. Both studies found an increased risk of CSCC from Cs use.

6.2.4 Infections

The main evidence of an involvement of infectious agents in skin carcinogenesis concerns cutaneous human papilloma virus (HPV). HPVs are small DNA viruses, belonging to the herpes virus group, and are subdivided in five subgroups (alpha, beta, gamma, mu, nu). They are highly host-specific and show a strict tropism for keratinocytes found in skin, oral mucosa, and anogenital mucosa.[120] About 120 HPV genotypes have been described so far and more are expected to be detected.[121] HPV is generally accepted as a cause of cervical cancer.[122] The involvement of HPV in CSCC development was first demonstrated in Epidermodysplasia verruciformis (EV) patients (see *Molecular mechanisms and inherited skin cancer susceptibility* below). The HPV types usually found in EV patients belong to the beta-HPV subgroup and are often referred to as EV-HPV. Of these, primarily HPV-5, but also 8, 14, 17, 20, and 47 are associated with cutaneous malignant conversion in said patients.[120]

No specific high-risk HPV has been linked to risk of CSCC in neither the general population, nor in OTRs.[120, 121, 123, 124] Studies of the association between HPV and NMSC in non-EV patients are further challenged by the presence of cutaneous HPV infection from early in life in virtually all individuals, the low number of viral DNA copies in skin cancer lesions, and the weak malignant transforming potential of cells *in vitro*.[121, 123] Although an association has not been established, there are some arguments in favor of a role for HPV in CSCC development post-transplantation. Firstly, the prevalence of viral warts in OTRs rises steadily after transplantation and the warts are located in sun exposed skin similar to CSCC.[125] Secondly, HPV DNA and EV-HPV DNA have often been detected in NMSC and precancerous lesions of OTRs.[124, 126-129] Thirdly, the presence of HPV infection and the viral load have been reported to be higher in skin tumors from OTRs than from the non-transplant population in some studies.[127, 128, 130] Lastly, some serological evidence of an association between HPV infection and risk of skin cancer has also been presented.[131] Nonetheless, there are some studies contradicting these findings.[132, 133] The molecular mechanisms underlying the malignant potential of HPVs are not entirely clear. The early (E) proteins E6 and E7 have been shown to have transforming properties and may inhibit apoptosis and DNA repair following UVR exposure.[121, 123] Furthermore, some HPVs are activated by increased levels of p53 and cytokines that are induced by UVR.[123] This indicates that HPV may promote the persistence of UV-damaged keratinocytes.[123]

The possible involvement of other herpes group viruses such as herpes simplex virus (HSV), cytomegalovirus (CMV), varicella zoster virus (VZV), and Epstein Barr virus (EBV) in CSCC development has barely been investigated before. Three experimental studies have investigated the presence of HSV, CMV and EBV DNA in skin lesions.[134-136] In one of these studies the presence of human herpes virus 6 and HSV-1 in skin lesions from 109 immunocompetent and 25 immunosuppressed individuals was analyzed.[134] It was found that immunocompetent persons infected with HSV-1 were at a 6-fold increased risk of CSCC compared to non-infected persons. There was no statistically significant difference between HSV-1 infected and non-infected immunosuppressed individuals. In a study on Greek immunocompetent patients, neither HSV-1, HSV-2 or EBV DNA could be detected in NMSC.[135] However, they found a high prevalence of CMV DNA in CSCC lesions. In contrast, in a small Swedish study on four heart transplant recipients (HTRs), EBV DNA was detected in 10 out of 15 CSCC/CSCC in situ.[136]

Bacterial, fungal, and hepatitis infections have not been investigated in association with risk of CSCC.

6.2.5 Human leukocyte antigens

Human leukocyte antigens (HLA) are antigen presenting proteins located on the cell surface. They are gene products of the highly polymorphic major histocompatibility (MHC) gene complex located on chromosome 6. HLA proteins are divided in class 1 (HLA-A, -B, and -C), which presents intracellular derived antigens, and class II (HLA-DR, -DP, and -DQ), which presents extracellular derived antigens. HLA together with a foreign antigen, for example from microorganisms, transplanted organs or tumours, triggers an immune response to eliminate the discovered threat.

The importance of matching HLA between recipient and donor with regard to graft- and patient survival is well established.[137, 138] Studies on the effect of HLA-matching on the risk of CSCC have been somewhat inconsistent. The majority of studies found no association between number of HLA-A, -B, -DR, or total number of mismatches and risk of CSCC post-transplantation.[55, 56, 58, 96, 139, 140] One case-control study from the Netherlands found an increased risk of CSCC with HLA-mismatches of B type but not of A or DR-type.[116]

Since HLAs are closely involved in creating immunity against foreign antigens it has been suggested that specific HLA types may be more or less efficient in provoking an immune response to malignant cells.[141] Indeed, studies have found both protective (HLA-A11, -DR4) and deleterious (HLA-A3, -A11, -B27, -DR1, -DR7, -DR homozygosity) associations with specific HLA types.[142] HLA-DR1 has been found to be related to developing single or multiple skin cancers in immunocompetent individuals.[142, 143] In OTRs, HLA-B27 was reported to be associated with risk of CSCC in two studies[144, 145] but not in three others.[96, 143, 146] Similarly, HLA-A11 was positively associated with NMSC and CSCC in two studies,[139, 146] negatively associated with

CSCC in one study,[145] and not associated with risk of CSCC/NMSC in three studies.[96, 143, 144] Results on HLA-DR7[58, 96, 144] and HLA-DR homozygosity[58, 96, 116] are also inconsistent. Hence, these studies have been contradictive and often challenged by a small size[143-145] and multiple testing due to the extensive polymorphisms of HLA. Furthermore, results may also be difficult to replicate due to population differences in HLA genotypes and environmental factors.

6.2.6 Other factors associated with organ transplantation

Other factors related to organ transplantation that may affect the risk of CSCC directly or indirectly, through an increased level of immunosuppression, are type of organ transplanted, number of transplantations and rejection episodes, donor vital status, donor age and sex, and cause of organ failure. It has been suggested that the immunogenicity of heart transplants is greater than that of kidney transplants and that liver transplants are the least immunogenic. This may affect the immune system both directly and indirectly. Additionally, since HTRs decease upon graft rejection and KTRs merely return to dialysis, it is believed that physicians are more generous with immunosuppressive drugs to HTRs, which may also influence CSCC risk indirectly. Two large cohort studies have reported an increased risk of CSCC in HTRs, of which one study[140] also found a decreased risk in liver transplant recipients, compared to KTRs.[96, 140] These finding were not replicated in one large[94] and one small[109] cohort study, and in one case-control study[147]. Neither number of transplantations,[55, 56, 96] number of rejection episodes,[55, 58, 96, 115, 116] nor donor vital status[56, 96, 140] have been associated with risk of CSCC in previous studies. Diabetic nephropathy, compared to other causes of kidney failure, has been related to a decreased risk of CSCC in some prior studies[95, 140] but the cause of organ failure has not been related to CSCC risk in other studies.[55, 56, 112]

6.2.7 Sunlight exposure and constitutional characteristics

Sunlight exposure (i.e. ultraviolet radiation (UVR) from the sun) is the most important risk factor for both CSCC and CMM.[148-150] UVR wavelengths range between 100 and 400 nm and are broadly categorized in UVA (320-400 nm), UVB (280-320 nm) and UVC (100-280 nm). UVB is more potent in inducing sunburn than UVA, but UVA penetrates deeper in the skin than does UVB.[151] UVR induces malignant transformation of keratinocytes and melanocytes by several mechanisms, including direct mutagenic effects on DNA, stimulation of cutaneous cells to produce growth factors, suppression of cutaneous (and systemic) immune defense, and production of reactive oxygen species that cause DNA damage and inhibit apoptosis.[152-154] The impact of sun exposure on risk of CSCC and CMM is demonstrated by the extremely high incidences in Australia, where the mainly fair-skinned population receives very high doses of ambient UVR.[5, 6, 155] Moreover, many epidemiological studies have presented evidence on the causal role of sunlight exposure in studies of the incidence of CSCC[47] and CMM[85, 156, 157] by latitude gradient, studies of migrants from high to low-latitude countries,[47, 68, 156] and in studies estimating the sunlight exposure in questionnaires and

interviews (CSCC[47, 158, 159], CMM[23, 68, 74, 75, 91, 157, 159, 160]). Although CSCC and CMM are both highly associated with sunlight exposure, different exposure patterns seem to be important in their etiologies. Increasing cumulative hours of sunlight exposure is associated with an increasing risk of CSCC.[149, 158, 161] This is also reflected by the anatomical distribution of CSCC on chronically sun exposed sites.[150, 155] CMM is more closely related to intermittent sunlight exposure and sunburns, both in childhood and in adulthood, but not to occupational sunlight exposure.[23, 91, 157, 160]

An increasingly popular source of UVR is artificial exposure from use of sunlamps or sunbeds. The emission of UVR from these devices is equally high or higher than the emission from midday sun in south Europe.[151] Most sources of artificial UVR consist mainly of UVA but also, to a smaller extent, UVB.[151] In 1992, IARC classified UVB and UVA radiation, as well as "use of sunlamps and sunbeds" as "probable carcinogenic to humans".[148] Use of sunbeds or sunlamps in association to CMM and CSCC has been investigated several times previously and pooled estimates have been presented by Gallagher *et al.*, 2005,[162] and IARC[151]. In these reports, ever use of sunbeds was positively associated with CMM with a RR of between 1.15 to 1.25, the risk was further increased if the exposure occurred early in life (RR 1.69 to 1.75). The evidence for an association with CSCC is limited but also suggests an increased risk in ever users of sunbeds that also increases with younger age at exposure.[151, 163]

Not only is the exposure from the sun important in the etiology of CSCC and CMM, the risk is also dependent on the individual susceptibility to the exposure. Constitutional characteristics, such as fair skin, skin reaction to sun (skin type, see **Table 5**), blond or red hair, and light eye color have been associated with both risk of CSCC[17, 47, 164, 165] and CMM[68, 74, 75, 165-167]. In a meta-analyses on CMM risk reported by Gandini *et al.*, 2005,[167] skin type I, fair skin color, and blond hair color were all associated with a 2-fold increase in risk, red hair color increased the risk 3.6 times, and light eye color increased the risk 1.6-fold, compared to skin type IV, dark skin color, and dark hair and eye color, respectively. Red hair color has consistently been associated with risk of CSCC.[17, 47, 164] The association with blond hair and light eye color has been less consistent.[17, 47, 164]

Table 5. Skin phototype according to Fitzpatrick classification.

Skin type	***Typical features***	***Tanning ability***
I	Pale white skin	Always burn, never tans
II	Fair skin	Burns easily, tans poorly
III	Darker white skin	Tans after initial burn
IV	Light brown skin	Burns minimally, tans easily
V	Brown skin	Tans darkly easily, rarely burns
VI	Dark brown or black skin	Always tans darkly, never burns

For CMM, the number of common and atypical (dysplastic) naevi is also a potent risk factor.[168, 169] In a meta-analysis of 46 studies published in 2005,[168] 101-120 common naevi were associated with an almost 7-fold (95% CI, 4.6-10.3) increased risk of CMM compared to <15 naevi. Presence of five atypical naevi increased the risk of CMM 6.4-fold (95% CI, 3.8-10.3), compared to having no atypical naevi.[168] Similar results were found in a pooled analysis of 15 case-control studies.[169]

There is no clear relationship between sunlight exposure and IMM.[26, 159, 170] In contrast to CMM, the incidence of IMM has been stable or decreased, as described earlier, and there is no latitude gradient.[26, 148] Primarily light iris color, but also other constitutional characteristics, have more consistently been associated with risk of IMM.[77, 170]

6.2.8 Molecular mechanisms and inherited skin cancer susceptibility

CSCC and CMM develop as a result of accumulated DNA damage in keratinocytes and melanocytes. This DNA damage ultimately leads to enhanced and uncontrolled cell proliferation, impaired DNA repair, and/or prevention of normal cell death (apoptosis).[153, 171, 172] In many CSCC lesions, but rarely in CMM lesions, UVB fingerprint mutations are found in target genes such as p53.[153, 173] p53 plays an important role in tumour prevention and is implicated in inhibition of tumour cell growth, cell-cycle arrest, differentiation, and apoptosis.[153, 174]

The effect of sunlight exposure-induced DNA damage in the skin is modified by variations (polymorphisms) in genes that affect the defensive response of the skin to UVR, for example skin pigmentation or "tanning". Skin pigmentation through melanin production is partly dependent on the actions of α-melonocyte-stimulating hormone (α-MSH) on its receptor, melanocortin-1 receptor (MC1R). Fair-skinned and red-headed people often carry polymorphisms in MC1R that reduces the activity of the receptor and thereby decreases the production of melanin and consequently the protection against UVR.[172, 175-177]

Patients suffering from Xeroderma Pigmentosum have a dramatically increased risk of CSCC, BCC and CMM.[153, 178] These patients lack one of the enzymes involved in nucleotide excision repair (NER).[178] NER therefore seems to be the main repair mechanism of UV-induced DNA damage.[153] Indeed, pyrimidine dimers, the UVB fingerprint DNA mutations, are repaired by NER.[153, 173]

Family history is the strongest risk factor for CMM.[171, 179] In Sweden, the standardized RR of CMM in individuals with one first degree kin with CMM has been reported to be between 2.4 and 3-fold increased, compared to the general population.[179] Nevertheless, CMM is rarely due to the presence of identifiable, inherited mutations in high penetrant genes. The proportion of melanomas caused by these gene mutations is unknown but is estimated to be less than two percent.[171, 180] So far, only two high penetrant genes involved in the cell cycle control have been identified in familial susceptibility to melanoma, CDKN2A (p16)[181, 182] and, less frequently, CDK4[183].[171, 172, 180] In Sweden, about 25 families have been identified, all with the same CDKN2A germ line mutation

(Ins 113 Arg) in chromosome 9p21.[184, 185] Furthermore, a locus on chromosome 1p22 has been found to be strongly linked to melanoma susceptibility but the responsible gene has not yet been identified.[186] There is also evidence for a susceptibility locus for IMM mapped to chromosome 9.[187]

CSCC is not strongly associated with a hereditary disease. However, patients with Epidermodysplasia Verruciformis (EV) suffer an increased risk of CSCC that often occurs early in life.[120] EV is a rare autosomal recessive disease that is classified as a primary deficiency in innate immunity to specific human papillomaviruses (HPV) (discussed in *Infections* section).[120] These patients get infected with HPV early in life and about one third of the patients develop CSCC.[188] The risk of malignant conversion is dependent on type of HPV. Two susceptibility genes have been identified for EV, EVER1 and EVER2, which encode transmembrane channel-like proteins located in the endoplasmic reticulum.[189] The link between these proteins and susceptibility to HPV infection is not entirely clear.[120]

6.2.9 Other risk factors

Arsenic exposure, mainly from contaminated ground water, is associated with risk of CSCC.[190] Arsenic accumulates in the skin and enhances the effect of UV exposure.[190] Chronic wounds and scar tissue from burn injuries have in the past been proposed to increase the risk of CSCC but recent large cohort studies does not support a causal association.[191-193] A diet consisting of vegetables has been reported to decrease the risk of CSCC.[194] This has been proposed to be mediated by antioxidant defense against UV-induced DNA damage in the skin. Similarly, a diet high in n-3 fatty acids has been reported to decrease risk of CSCC, possibly through an effect on the UV-induced inflammatory response.[195] An intervention study of beta-carotene supplementation in relation to the incidence of CSCC revealed no effect of supplementation.[196] In contrast, a high, compared to a low, consumption of alfa-carotene, beta-carotene, cryptoxanthin, lutein, and lycopene was found to decrease risk of CMM in a large case-control study.[197] The risk of CMM was also reduced with intake of vegetables and fruits in this study. However, these findings have not been replicated in other case-control studies.[68, 198] A meta-analyses[199] and a pooled analyses[200] of oral contraceptive use found no relation to risk of CMM. Similarly, hormone replacement therapy has not been associated with risk of CMM.[66, 71, 88]

7 SPECIFIC AIMS

The overall aim of this thesis was to increase our understanding of the etiology of CSCC, CMM, MIS, and IMM with regard to tobacco use, BMI and organ transplantation.

Specifically, the following research questions were addressed:

Study I and II:

- Is current or previous tobacco use associated with risk of CSCC, CMM, MIS, and IMM, compared to never use?

- Is high dose and long duration of tobacco use associated with risk of CSCC, CMM, MIS, and IMM, compared to low dose and short duration of tobacco use?

- Is underweight, overweight or obesity related to risk of CSCC, CMM, MIS, and IMM, compared to normal weight?

Study III and IV:

- Is the risk of CSCC post-transplantation primarily conferred by an overall drug load or is the risk differentially conferred by specific immunosuppressive agents?

- Is increasing dose and longer duration of immunosuppressive treatment associated with an increased risk of CSCC in organ transplant recipients?

- Is the risk of CSCC in organ transplant recipients associated with an overall immunosuppressive state, as measured by infectious load?

- Is the risk of post-transplant CSCC related to bacterial, viral or fungal infections, HLA type or mismatches, number of transplantations, type of organ transplanted, number of rejections, donor characteristics, or cause of organ failure?

8 SUBJECTS AND METHODS

8.1 SWEDISH REGISTRIES AND LINKAGE

8.1.1 National registration numbers

The individually unique ten digit national registration numbers were introduced in Sweden in 1947. The number originally consisted of nine digits but an extra digit was added in 1967 when the system was computerized. The first six digits correspond to the person's birth date and after a hyphen follows a serial number of three digits and lastly a check digit. The national registration numbers are used extensively in the Swedish society, for example by authorities, health care, banks and insurance companies. Consequently, these numbers can be used to link registries to one another and thereby collect information on one individual from several sources.[201]

8.1.2 Swedish National Patient Register

The Swedish National Board of Health and Welfare started to collect information in 1964 on hospital discharges in Sweden to the former In-Patient Register. The coverage of Swedish in-patient care reported gradually increased thereafter to include 60% in 1969, 85% in 1983, and 100% from 1987 and onwards. The register was later renamed to the National Patient Register when national registration numbers were allowed to be included in the register.[202]

Information recorded in the National Patient Register includes patient related characteristics (national registration number, sex, age, county), health care related information (hospital, clinic), administrative information (date of admission and discharge), and medical data (discharge diagnoses and surgical procedure codes). Diseases are recorded according to the current revision of the WHO's International Classification of Diseases (ICD), and surgical procedures are coded according to either of the two editions of "Classification of Operations and Major Procedures" published by the National Board of Health and Welfare.

The completeness of the data has been evaluated previously and found to be of generally high quality. Approximately 98% of in-patient episodes report valid national registration numbers and only about 1.2% of the episodes lack a main diagnosis.[202] However, the discharge code data has been found to hold a somewhat lower quality with incorrect codes in approximately 10-12%. Surgical procedure codes have been found to be correct in 98% but with an underreporting of approximately eight percent.[203]

8.1.3 Swedish National Cancer Register

The Swedish National Cancer Register was founded in 1958 and is administered by the Swedish National Board of Health and Welfare. Primarily, information is collected by

six regional cancer registries, all information is then gathered annually and published by the National Cancer Register. Reporting to the regional cancer registries is mandatory by law for both the diagnosing physician and the pathologist, ensuring registration of 96-98% of all incident cancers with a histological verification of approximately 99% of reported cases.[19, 204-206]

All malignancies and some specifically defined benign tumors and precancerous lesion are reported to the Swedish National Cancer Register. Information available includes national registration numbers, sex, domicile, hospital, site of tumor, histological type, stage, and date and basis of diagnosis. Classification and site of tumors are recorded according to the current ICD revision together with a translation to the seventh revision of ICD for historical comparability.

Skin cancers are reported as either malignant melanoma or nonmelanoma skin cancer. Almost all registered nonmelanoma skin cancers are CSCC since the registration of basal cell carcinoma was first initiated in 2003 (see *Methodological considerations*).[18]

8.1.4 Swedish Cause of Death Register

Cause of death registration was implemented in Sweden already in 1749 but did not become complete and internationally compatible until 1951 when reporting routines were changed and the sixth revision of ICD was introduced for the classification of death causes. The registry contains information on primary and contributory causes, date of death, and national registration numbers. The Cause of Death Register is essentially complete and the ICD coding for causes has generally been found to be of satisfactory quality.[207]

8.1.5 Swedish Register of the Total Population

Statistics on population changes emanate from the Register of the Total Population held by Statistics Sweden. Publication of population changes started in 1968 but registration is available from 1961. Emigration has been registered since 1969. The reliability of the register is generally good but there is some under- and overestimations due to unregistered immigration and emigration.[208]

8.2 STUDY DESIGN

8.2.1 Study I and II

8.2.1.1 Construction Workers Cohort

Study I and II were performed in a large cohort of 386 000 Swedish construction workers. The Construction Industry's Organization for Working Environment, Safety and Health (Bygghälsan) provided out-patient medical services to construction workers all over Sweden from 1969 through 1993. The organization was a joint venture launched by the relevant trade unions and the Swedish Construction Employers' Association. The basic units were stationary or mobile clinics, typically staffed by a physician and a few nurses. The main activity was preventive health check-ups, offered to all blue- and white-collar employees in the building industry through regular (every second year during the first years and every third year thereafter) personal invitations and through visits to, or advertisements at, virtually all major building sites. Although the program was voluntary, 85-90% of eligible workers participated at least once.[209] On average, each cohort member underwent three health check-ups but we only used information from the first visit in the analyses.

8.2.1.2 Exposure information

Beginning in 1971, the collected information was stored in a computerized register. Exposure information was collected through several sources and differed somewhat during the study period (**Figure 6**). In 1971-1975 information was mainly collected by two different forms; one *self-administered questionnaire*, containing extensive information (~200 items) about occupational exposures, exposures to tobacco products, physical and mental symptoms, diagnosed diseases, and use of prescribed drugs; and one *staff-administered form* containing job code information and basic medical measurements such as pulse, blood pressure and anthropometric measures.

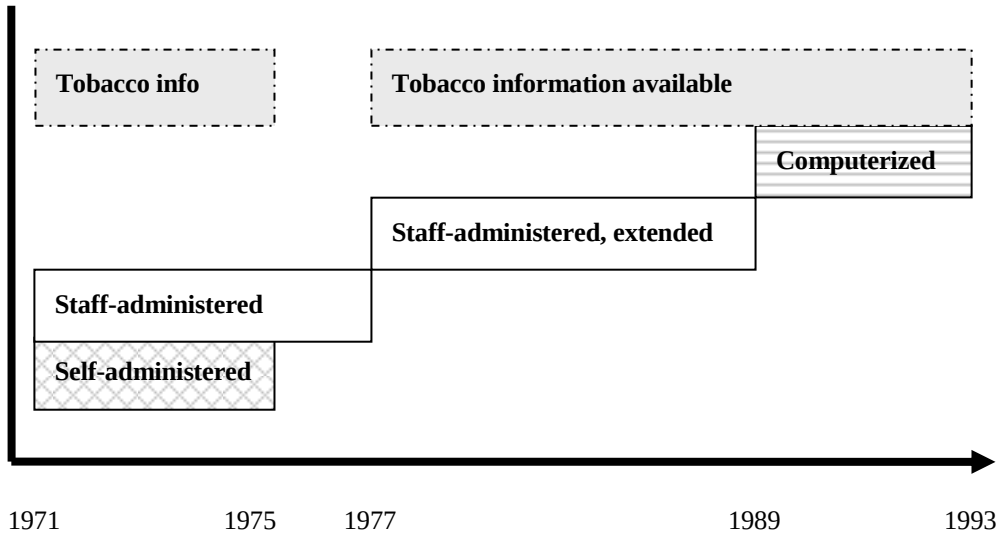

Figure 6. Methods for exposure collection 1971-1993 by the Construction Industry's Organization for Working Environment, Safety and Health (Bygghälsan).

The self-administered questionnaire was double-checked by a nurse during this period to prevent misunderstandings and inconsistencies. From 1975 to 1977, the self-administered questionnaire was not in use, and from 1978 the staff-administered form was expanded to include all information collected earlier in even more detail. In 1989, the questionnaire was computerized.

8.2.1.3 Sunlight exposure assessment - Study II

Information on sunlight exposure was not collected from the construction workers, however, occupational sunlight exposure can be estimated from job task codes. During 1971-1985 codes for 200 different job tasks were recorded in the construction workers cohort, but after 1985 the job tasks were divided in only 90 categories. These latter job tasks corresponded to one or several of the former codes. Using these job task codes, an experienced industrial hygienist (N. Hallin) from the construction industry assessed the occupational exposure to sunlight from outdoor work. In the latter code system, the average level for any group of codes was used in the exposure assessment. Furthermore, during 1971-1985 workers registered information on both previous and current job tasks, but after 1985 there is only information the current job task. By examining the data from workers who had registered before 1985, it was found that few changed their duties and that 96.3% were equally exposed to sunlight in previous and current job tasks.[210] Hence, only current job task information was used in the analyses. The sunlight exposure was classified into four categories with increasing exposure scores from null to three, according to the specified criteria in **Table 6**.

Table 6. Criteria for the classification of sunlight exposure according to job tasks and examples of some typical job tasks within each exposure group. Adapted from Håkansson *et al.*[210]

Exposure category	Amount of outdoor work	Typical job tasks
0	Never or seldom works outdoors.	Management, electrician, painting, pipe fitting, building repair work
1	Works outdoors to some extent during the workday, some shade from for example building and trees occur.	Frame workers, repair and maintenance work, excavator operating, crane operating, repair of machines and equipment
2	Works outdoors to a great extent during the workday, some shade from for example building and trees occur.	Formwork moulding, concreting work, concrete reinforcement work, fronting, scaffolding
3	Works outdoors almost the entire workday, the whole year or in summertime, mostly unprotected from sunlight.	Roofing, roof paper covering work, plate covering of walls, road construction machine operating, paving

8.2.1.4 Quality of exposure data

The quality of the registered data has been investigated by internal comparisons previously.[211] Body height, that is not expected to vary over time, and information on smoking were chosen as indicators of data consistency. Information on height was missing in 0.1%. Divergence of less than one centimetre was found in 79% and less than four centimetres in 98.5% of the height measurements. Missing data on smoking duration was found in 1.3% of current smokers and 1.4% of previous smokers. Perfect concordance between reports on smoking status, typically two to three years apart, was found in 89%. Inconsistencies (e.g. people claiming to be current or previous smokers in the first visit but in a later visit claimed to never have smoked) were found in 2.6%.

8.2.1.5 Study subjects and follow-up

The first registered health check-up defined entry into the cohort.

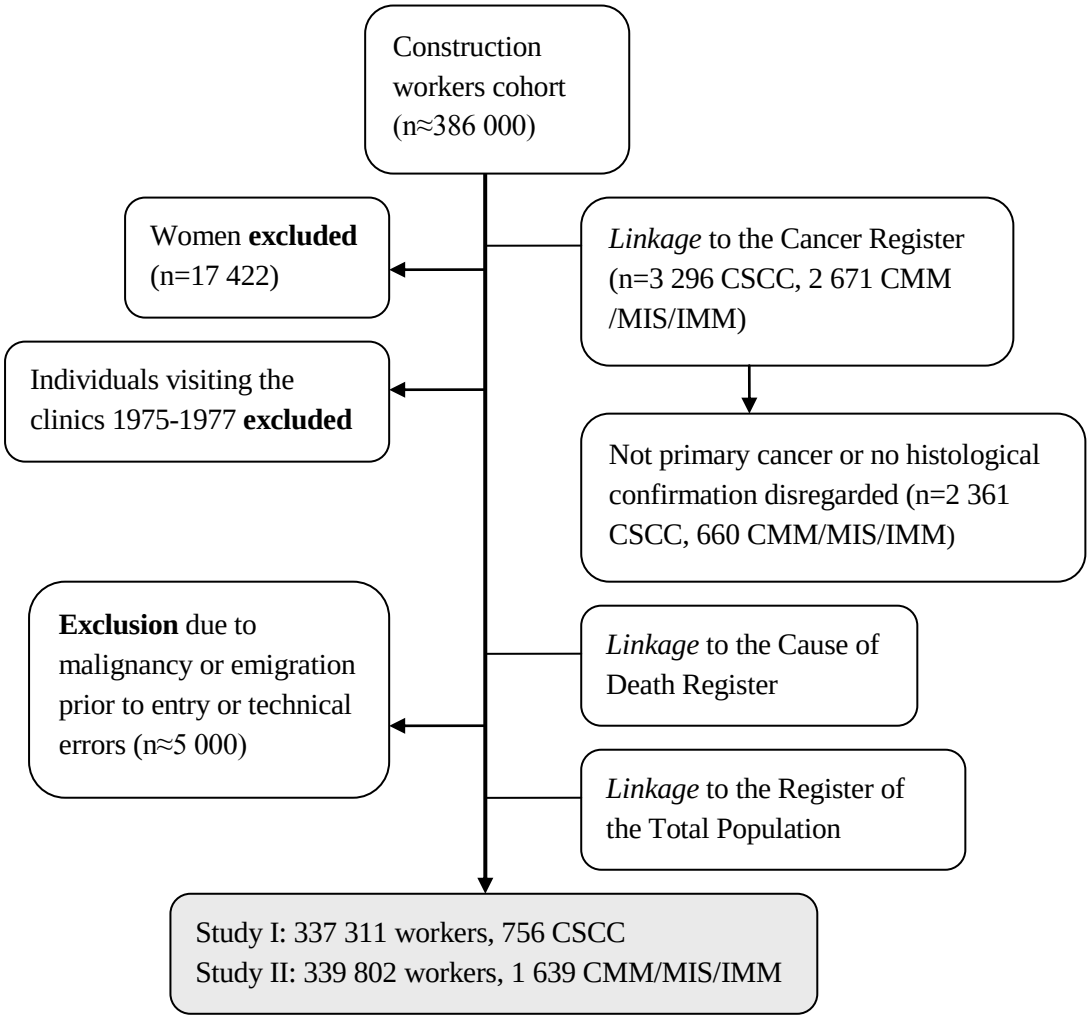

Figure 7. Flowchart for selection and follow-up of study subjects in Study I and II.

The construction workers were followed-up by record linkage to the National Cancer Register, Cause of Death Register, and the Register of the Total Population, from date

of entry until CSCC or CMM/MIS/IMM diagnoses, death, emigration or end of follow-up 31st December 2000 (Study I) and 31st December 2004 (Study II), whichever occurred first (**Figure 7**). As no information on tobacco use was collected in 1975 to 1977, construction workers registered in this period were excluded. Moreover, since less than five percent of the cohort consisted of women, we restricted the study to male subjects. Lastly, workers with a history of cancer (n=1 232), workers that emigrated prior to entry into the cohort (n=2 898), and workers with erroneous personal identification numbers (n=570) were excluded. After these exclusions we were left with 337 311 construction workers for Study I and 339 802 for Study II.

3 296 CSCC and 2 671 CMM/MIS/IMM occurred during follow-up to 31st December 2000 and 2004, respectively. Since only cases of first and histologically verified malignancies were included (**Figure 7**), 756 CSCC and 1 639 CMM/MIS/IMM finally constituted the case population in Study I and Study II, respectively.

8.2.2 Study III and IV

8.2.2.1 Swedish Solid Organ Transplantation Cohort

Transplantations are only performed in four public University hospitals (Karolinska, Sahlgrenska, Uppsala, and Malmö/Lund) in Sweden. Thus, the registration of organ transplantation in the National Patient Register is population-based. Two of these hospitals reported to the National Patient Register already from 1970 but the other two did not start reporting until 1972, therefore some organ transplantations (approximately 50) were not included in the study.[94, 212] The cohort was assembled by selecting all organ transplant patients from the National Patient Register (n=6 457). Date of the first transplantation marked entry into the cohort and patients were followed (by linkage to the relevant registers) to cancer diagnoses, death, emigration or end-of follow-up 31st December 1997, whichever occurred first. After exclusion of patients with a history of cancer (n=77), cancer reported within 30 days post-transplantation (n=179), patients with unknown transplantation codes (n=258) or mismatching transplantation dates (n=12), the cohort consisted of 5 931 OTRs (**Figure 8**).[94]

8.2.2.2 Identification of CSCC cases and controls

By linkage to the Swedish National Cancer Register we identified all patients that developed CSCC as a first cancer diagnosis during follow-up (1970-1997). Controls were randomly chosen from the Swedish solid organ transplantation cohort and individually (1:1) matched by age (±5 years) and calendar period of (±5 years) transplantation. The controls were also required to be alive and free from cancer at the time of the corresponding case CSCC diagnosis. Nineteen patients (3.9%) were excluded since they had an unregistered prior transplantation or because they died before the end of follow-up, and two patients (0.4%) developed a cancer that was not registered in the Swedish Cancer Register. From all living cases and controls (n=233), we requested a written, informed consent, which all but 14 patients (6%) approved. Of the 451 remaining patients, 53 patients (12%) were lost to follow-up since their medical

records could not be located. After these exclusions, the study subjects consisted of 207 cases (88% of eligible cases) and 189 controls (84% of eligible controls) (**Figure 8**).

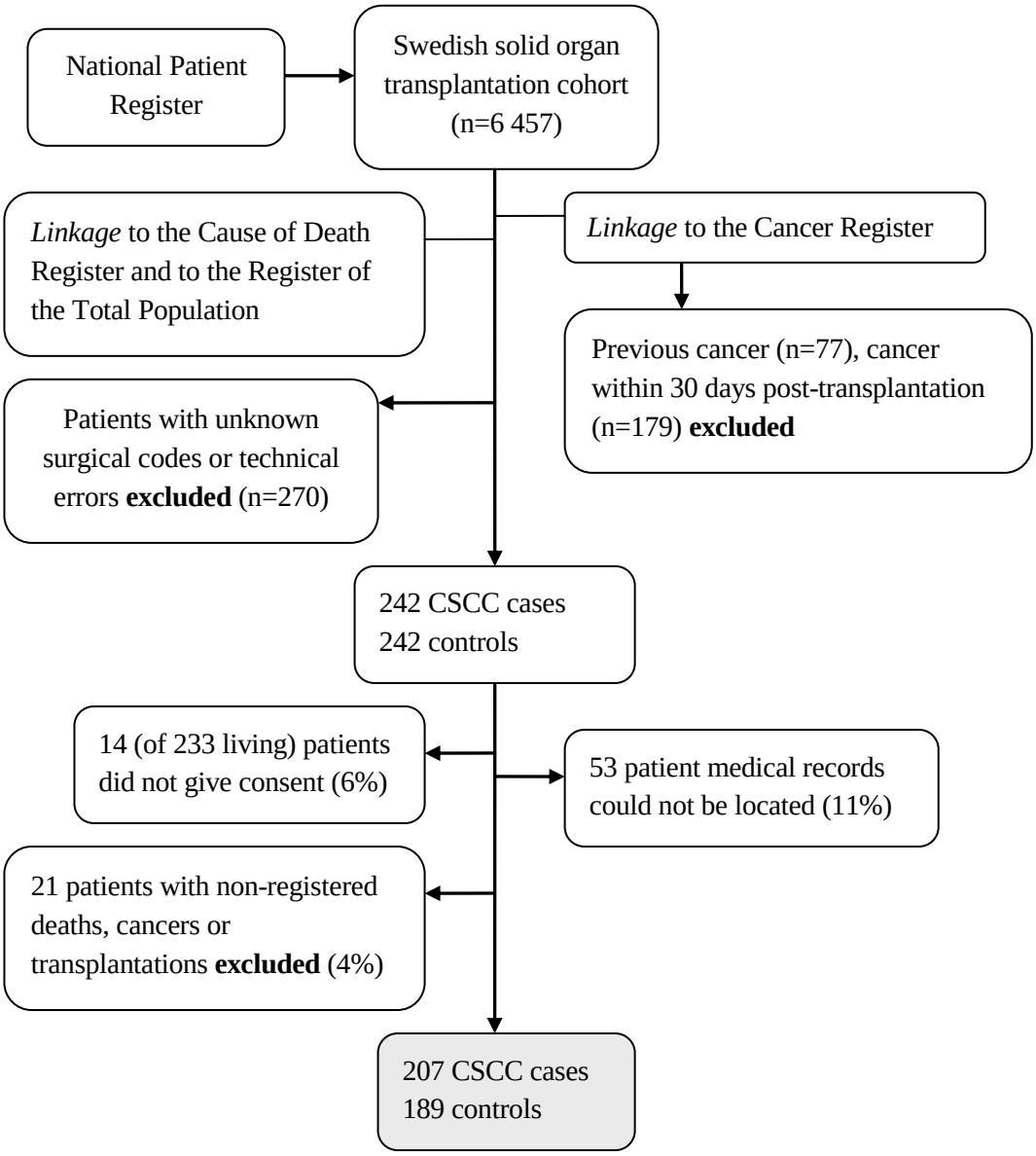

Figure 8. Flowchart for selection and follow-up of study subjects in Study III and IV.

8.2.2.3 Collection of exposure information

An extensive form was created and pilot tested for retrieving information from the patient medical records in a standardized manner. Data collectors were trained by and in continuous contact with J Adami (researcher), Å Odenbro and P Fernberg (PhD-students). Efforts were made to blind the data collectors for the case/control status of patients. Over 95% of the data was collected by four data collectors (the majority by Å Odenbro). The assembly of the data started at one of the four university hospital

transplantation centers and continued at regional hospitals when needed. Data collected included sex, age, HLA, and serology pre-transplantation of recipient and donor, cause of organ failure and time in dialyses treatment (kidney recipients), rejection episodes, vital status of donor, date of infections with viral, bacterial and fungal species. In addition, we recorded the daily doses, administration forms (oral/intravenous/ intramuscular/subcutaneous), and all dose changes with dates for treatment initiation and termination of the following drugs: Aza, CsA, CsA microemulsion, Cs (prednisolone, prednisone, methylprednisolone, hydrocortisone, betamethasone, and cortisone), tacrolimus, mycophenolat mofetil, sirolimus, cyclofosphamide, ATG, ALG, and OKT-3.

8.2.2.4 Interpretation of clinical and missing data

Generally, all information included in these studies was available in the patient medical records, however, some rules were defined for the interpretation of clinical and missing data. Inevitably, some drug dose changes could not be tracked to a specific date, the median date between the last known date of the old dose and the first known date of the new dose was then adopted. All HLA antigen splits were standardized to their broad HLA antigen. A HLA mismatch was defined as a HLA phenotype present in the donor but absent in the recipient.[139, 145] When only one antigen could be identified for a given locus, homozygosity was assumed.[139] HLA typing method differed between laboratories, time periods, and HLA loci, and included serological or genomic typing. Information on typing method was not collected. A bacterial infection was defined as either a positive culture with >100 000 bacteria/ml or antibiotic treatment of symptoms. A herpes group virus infection was defined by clinical diagnoses as noted in the medical records, with or without treatment or serological evidence of seroconversion or a significant increase in specific IgG antibodies. Hepatitis infection was determined by serological evidence. Fungal infections included infections with yeast infections (mainly Candida Albicans), Pneumocystis carinii and Aspergillus species.

8.3 STATISTICAL ANALYSES

Data management and analyses of the data in this thesis were performed using Stata Statistical Software, release 8.2 or later (StataCorp. *Stata Statistical Software*: College Station, TX, U.S.A.).

8.3.1 Study I and II: Cox proportional hazards model

The Cox proportional hazards model is commonly used in medical research. It provides estimates of the incidence rate ratio (IRR, from here on denoted as RR), also termed hazard ratio, using the maximum partial likelihood method. The Cox proportional hazards model studies the instantaneous event rate at time t, given that the individual has remained free of the event until that time. While the model allows the hazards to vary with time and does not make any assumptions about their shape or direction, it assumes proportional hazards over time, i.e. the RR of an exposed individual compared to an unexposed individual should be equal at all time points. This is a strong assumption and its appropriateness should always be assessed and properly adjusted for when it does not hold.[213, 214]

The effect of tobacco use and BMI was investigated in relation to occurrence of CSCC in Study I and occurrence of CMM, MIS and IMM in Study II. The distribution of BMI was categorized according to the WHO's criteria: underweight <18.5 kg/m^2, normal weight 18.5-24.9 kg/m^2, overweight 25-30 kg/m^2, and obesity >30 kg/m^2. Since there were few cases in the extreme categories of BMI in Study II, data was analyzed in two categories, underweight/normal weight and overweight/obese. The distribution of age was categorized in eight groups, each encompassing a five-year range. When assessing the daily quantity (g) of cigarette and cigar tobacco smoked by each worker, cigarettes were assumed to contain 1 g of tobacco and cigars 6 g of tobacco. Pipe smokers and snuff users reported the amount of tobacco (g) used every week.

Tobacco use status, quantity, duration, recency of cessation, as well as BMI was established from the information given at entry into the cohort. In calculating the accumulated quantity smoked (duration X quantity) in Study II, the duration included the study follow-up time for current smokers.

RRs and 95% CIs were estimated using Cox proportional hazards model with time in years as the time scale. The RR was adjusted for age in Study I, and for age, BMI, occupational sunlight exposure, and birth cohort in Study II. The relevant exposures were included as independent variables and their effects were estimated in separate models. Statistical significance was tested with likelihood ratio tests. Linear trends in risk were estimated by including "scored" categorized variables in the models. These scored variables were created by taking the mean of the continuous exposure in each category of the categorized variable. Biologically plausible interactions on the multiplicative scale were tested by including interaction terms in the model, statistical significance of interactions were assessed with likelihood ratio tests.

The data was analyzed both with cancer-censoring of cohort members only at the study-specific cancer diagnoses (CSCC in Study I and CMM/MIS/IMM in Study II) and with cancer-censoring at any cancer diagnoses. The previous method was used in the published manuscripts and since the results were unchanged this was not altered.

The proportional hazards assumption of the Cox model was analyzed by looking at the distribution of Schoenfeld residuals and by the related test of proportionality. When the assumption was not fulfilled a stratified model was fitted.

8.3.2 Study III and IV: conditional logistic regression

Logistic regression estimates the probability of a binary outcome as a function of one or more exposures, while controlling for other variables. The model provides estimates of the OR using the maximum likelihood method. For matched case-control studies, common logistic regression will produce biased estimates of the OR since one of the underlying assumptions of the model is violated. Conditional logistic regression, on the other hand, provides unbiased estimates of the ORs in matched case-control studies.

Before the initiation of analyses, we combined generically similar drugs and converted different administration forms to reflect equal bioavailability. All Cs medications were standardized to the corresponding prednisolone dose, CsA microemulsion was converted to represent CsA, and all orally administered drugs were transformed to correspond to 100% bioavailability (**Table 7**).

Doses of drugs were categorized according to the tertile distribution among controls, with never treatment as reference. Since all patients received corticosteroids as part of their treatment regimen, all corticosteroid variables were categorized according to the quartiles distribution among controls. To reflect a weighted total dose load in patients receiving all of Aza, CsA and Cs, we assigned reference values to the accumulated dose categories of each drug (reference=0, low=1, intermediate=2, high=3) and summed them up. A variable for the total number of infections was created by adding the numbers of herpes group virus infections, hepatitis, bacterial, and fungal infections. Infections during the last 30 days prior to CSCC diagnosis were censored to avoid reversed causality.

Correlations between variables were first tested using Spearman correlation coefficient (r_s).[215] ORs were estimated using conditional logistic regression models and served as measures of RRs. Exact logistic regression was used for exposure variables with low numbers of exposed cases and/or controls. Model building was based on purposeful selection criteria, relying on both statistical and biological relevance of factors considered.[216] Potential risk factors or confounders were entered in a multivariate model and their effects were tested with likelihood ratio tests. Thereafter all exposure variables were added, one at a time, to the multivariate conditional logistic regression model and tested accordingly for statistical significance. The final model consisted of sex of the recipient and total accumulated dose of Aza, CsA, and Cs, respectively. Analyses of combinations of drugs or drug doses in different treatment phases were not

adjusted for the total accumulated dose of the same drug. Effect modification, on a multiplicative scale, between two variables of Aza, CsA, Cs, sex, and age was tested by including an interaction term in the model. Linear trends in risk of CSCC were tested as described for Study I and II above, but using the median instead of the mean. Sensitivity analyses were performed for exposure variables when the proportion of missing information exceeded 15% (HLA-DR, age and sex of donor) by including all missing information in the extreme categories of the variable, one at a time, and re-analyzing the data.

Table 7. Algorithms for combining generically similar immunosuppressive drugs.

Generic name of drug administered orally	**Conversion factor used**	**Product comparable to:**
betamethasone	8.33	prednisolone
methylprednisolone	1.25	prednisolone
hydrocortisone	0.25	prednisolone
cortisone	0.20	prednisolone
prednisone	1.00	prednisolone
prednisolone	0.80	intravenously administered methylprednisolone (100% bioavailable)
cyclosporine	0.25	intravenously administered cyclosporine (100% bioavailable)
cyclosporine microemulsion	0.38	intravenously administered cyclosporine (100% bioavailable)
azathioprine	0.47	intravenously administered azathioprine (100% bioavailable)

Some cases and controls (n=71) lost their originally matched partner due to loss to follow-up or technical errors. These subjects were joined together in new pairs or entered into other risk sets if they fulfilled the original matching criteria (n=18). In order to use the information from additional subjects without a matched partner, we applied slightly revised matching criteria (±10 years for age and calendar time and <60 days difference in follow-up time) allowing another 34 participants to enter into existing risk sets. Analyses were performed both with and without the additional 52 participants and the results changed only marginally, therefore analyses including these subjects are reported.

9 RESULTS

9.1 STUDY I

During a total of 6 536 910 person-years of follow-up, 756 male workers developed CSCC as a first malignancy. The mean age at entry into the cohort was 34.2 years (range 14-82) and the mean age at CSCC diagnoses was 69.9 years (range 34.7-92.2). The cohort members were on average followed for 19.4 years (range 0-31.3). Fifty-eight percent of the subjects had ever smoked cigarettes, cigars or pipe and 28% had ever used snuff. Approximately 45% of the workers exclusively used one kind of tobacco, most commonly cigarettes (≈30%) and snuff (≈10%). The workers who used more than one tobacco product (≈25%) usually mixed cigarettes and snuff (≈14%) or cigarettes and pipe (≈5%).

The age-adjusted RRs of CSCC and 95% CIs for cigarette, cigar, and pipe smoking (snuff users excluded) analyzed together are presented in **Table 8**. There was no association between smoking tobacco and risk of CSCC. Compared to never tobacco users, the RRs of CSCC in both previous and current smokers were 1.0 (95% CI, 0.8-1.2). Furthermore, there were no trends in risk of CSCC present for increasing smoking tobacco dose or duration.

Table 8. Age-adjusted incidence rate ratios (RR) and 95% confidence intervals (CI) of cutaneous squamous cell carcinoma (CSCC) in relation to ever use, dose, duration, and recency of all smoking tobacco products analyzed together.

Exposure variable	No of cases (%)	Person-years accumulated	RR (95% CI)
Reference (never tobacco user)	209 (35)	1 920 810	1 (ref)
Any tobacco smoker			
Previous	141 (24)	710 190	1.0 (0.8-1.2)
Current	245 (41)	2 150 910	1.0 (0.8-1.2)
Total smoking tobacco dose (grams per week)			
≤10	222 (39)	1 410 290	1.0 (0.8-1.2)
11-15	112 (20)	1 119 380	0.9 (0.7-1.1)
>15	27 (5)	230 650	1.0 (0.6-1.4)
Years of tobacco use			
≤15	87 (15)	1 534 570	1.1 (0.8-1.4)
16-25	110 (19)	725 130	1.1 (0.9-1.4)
>25	188 (32)	563 890	0.9 (0.7-1.1)
Time since cessation of tobacco use (years)			
<10	74 (21)	487 210	1.0 (0.8-1.3)
≥10	66 (19)	197 480	0.9 (0.7-1.2)

Discrepancy in number of cases and person-years is due to missing information in dose and duration.

In **Table 9**, exclusive use of tobacco products in relation to risk of CSCC is presented. Again, tobacco smoking was unrelated to risk of CSCC. The RRs of CSCC in pure cigarette, cigar, and pipe smokers were 1.0 (95% CI, 0.9-1.3), 0.9 (95% CI, 0.4-1.7), and 0.8 (95% CI, 0.6-1.0), respectively, compared to never tobacco users. No trend in risk of CSCC was present with increasing number of cigarettes smoked per day or amount of pipe tobacco smoked per week.

Table 9. Age-adjusted incidence rate ratios (RR) and 95% confidence intervals (CI) of cutaneous squamous cell carcinoma (CSCC) in relation to exclusive use of cigarettes, cigars, pipe, and snuff, and mixed use.

Tobacco variable	No. of cases (%)	Person-years accumulated	RR (95% CI)
Reference (never tobacco users)	209 (28)	1 920 810	1 (ref)
Cigarette smoker	194 (26)	1 947 400	1.0 (0.9-1.3)
Cigarettes smoked/day			
<10	105	836 590	1.1 (0.9-1.4)
11-20	58	667 330	0.9 (0.7-1.2)
>20	26	405 710	0.9 (0.6-1.4)
Cigar smoker	9 (1)	42 000	0.9 (0.4-1.7)
Pipe smoker	80 (11)	358 200	0.8 (0.6-1.0)
Pipe tobacco (g) smoked/week			
<80	75	338 180	0.8 (0.6-1.0)
≥80	5	19 790	1.1 (0.5-2.7)
Snuff dipper	29 (4)	661 150	0.6 (0.4-0.9)
Years of snuff use			
<30	14	610 320	0.8 (0.5-1.4)
≥30	15	44 660	0.6 (0.3-1.0)
Mixed user	235 (31)	1 607 340	1.1 (0.9-1.3)

Discrepancy in number of cases and person-years is due to missing information on dose and duration.

Exclusive snuff use was negatively associated with risk of CSCC (RR 0.6; 95% CI, 0.4-0.9) and there was an indication of a decreasing risk with longer duration of snuff use. However, these results were based on relatively few cases.

BMI was not associated with risk of CSCC. Being underweight (BMI≤18.5 kg/m^2) and obese (BMI>30 kg/m^2) conferred a RR of 0.9 (95% CI, 0.3-2.7) and 1.0 (95% CI, 0.7-1.3), respectively (data not shown).

9.2 STUDY II

During a mean follow-up of 22.6 years (range 0.01-33.5), a total of 7 663 400 person-years accrued and 1 309 workers developed CMM, 267 workers developed MIS, and 63 workers developed IMM. The mean age at diagnoses of CMM, MIS and IMM was 56.2, 57.6, and 61.4 years, respectively. The distribution of tobacco products used has been described above. The mean BMI of the workers was 24.2 kg/m^2 and most of the workers were exposed to low or medium amount of occupational sunlight.

Table 10 presents the RR of all melanoma together and separately for CMM, MIS and IMM in relation to the combined effect of all tobacco smoking products (cigarette, cigar and pipe smoking, snuff users were excluded). Current smokers were at a 30-50% lower risk of developing all outcomes, compared to never tobacco users. Former smokers and smoking cessation within 10 years were related to a 20% reduced risk of CMM (95% CI, 0.6-0.9), but the risk of MIS and IMM was not affected. With increasing duration of smoking, the risk of CMM and MIS was reduced, and more than 20 years tobacco smoking duration conferred a statistically significant RR of 0.6, compared to never tobacco users. The risk of CMM, MIS and IMM also decreased with increasing quantity tobacco smoked per day, and this effect was even stronger in the analyses of the accumulated quantity (duration X quantity). In the highest category of accumulated quantity (>999 g or >50 pack-years) of smoked tobacco, the risk of CMM was reduced by 60% (95% CI, 0.3-0.6) and the risk of MIS was reduced by 70% (95% CI, 0.1-0.7), compared to never tobacco users. Similarly a high accumulated quantity of smoking tobacco non-significantly reduced the risk of IMM 80% (95% CI, 0.03-1.6), but this was based on few cases. The tests for linear trend in risk of CMM, MIS or IMM in relation to quantity and accumulated quantity of smoking tobacco were not significant.

The exclusive use of cigarettes, cigars, pipe and snuff, and mixed use in relation to risks of CMM, MIS and IMM are displayed in **Table 11**. Compared to those who had never used tobacco, pure cigarette smokers were associated with a 30% statistically significant reduced risk of CMM and MIS. The risk of IMM was not associated with pure cigarette smoking. Exclusive pipe smoking was related to a 40% decreased risk of CMM and IMM, and a 60% reduced risk of MIS, but the confidence intervals for MIS and IMM were not significant. Cigar smoking was unrelated to risk of CMM. Estimates relating quantity of cigarette tobacco smoked to risk of melanoma were similar to those on quantity of combined tobacco products smoked. Snuff use was associated with a significant and a non-significant 40% reduced risk of CMM (95% CI, 0.5-0.8) and MIS (95% CI, 0.4-1.1), respectively, compared to never tobacco users. This risk decreased with longer duration of snuff use (p_{trend}<0.01 for CMM and p_{trend}=0.08 for MIS). There was no association between snuff use and risk of IMM. Generally, analyses on risk of IMM were based on small number of cases and the results were therefore difficult to evaluate.

BMI corresponding to overweight/obesity (≥25 kg/m^2) was associated with a 1.3-fold increased risk of CMM (95% CI, 1.2-1.5), compared to underweight/normal weight (<25 kg/m^2) (data not shown). Risk of MIS or IMM was not associated with BMI.

Table 10. Adjusted incidence rate ratios (RR)[¶] and 95% Confidence Intervals (CI) of all malignant melanoma combined, cutaneous malignant melanoma (CMM), cutaneous melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM) in relation to ever use, dose, accumulated dose, duration, and recency of all smoking tobacco products analyzed together.[†]

	All Melanoma combined		**CMM**		**MIS**		**IMM**	
	No. of cases	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**
Never tobacco users	599	1-Ref	476	1-Ref	103	1-Ref	20	1-Ref
Smoking status								
Previous	236	0.8 (0.7-0.9)	183	0.8 (0.6-0.9)	40	1.0 (0.7-1.4)	13	1.1 (0.5-2.1)
Current	454	0.6 (0.6-0.7)	377	0.7 (0.6-0.8)	61	0.5 (0.3-0.7)	16	0.6 (0.3-1.1)
Dose of tobacco smoked/ day (g)*[§]								
1-5	124	0.8 (0.6-0.9)	97	0.8 (0.6-1.0)	20	0.7 (0.4-1.2)	7	0.9 (0.4-2.3)
6-15	279	0.6 (0.5-0.7)	223	0.6 (0.5-0.7)	42	0.6 (0.4-0.8)	14	0.8 (0.4-1.6)
>15	256	0.7 (0.6-0.8)	214	0.7 (0.6-0.8)	36	0.6 (0.4-0.9)	6	0.5 (0.2-1.3)
		$p_{(trend)} = 0.18$		$p_{(trend)} = 0.32$		$p_{(trend)} = 0.23$		$p_{(trend)} = 1.00$
Duration of smoking (y)[§]								
1-10	191	0.7 (0.6-0.9)	152	0.8 (0.6-0.9)	32	0.7 (0.4-1.0)	7	1.1 (0.4-2.8)
11-20	255	0.7 (0.6-0.9)	209	0.7 (0.6-0.9)	33	0.6 (0.4-0.9)	13	1.2 (0.6-2.5)
>20	237	0.6 (0.5-0.7)	193	0.6 (0.5-0.7)	35	0.6 (0.4-0.9)	9	0.4 (0.2-0.9)
		$p_{(trend)} < 0.01$		$p_{(trend)} < 0.01$		$p_{(trend)} < 0.01$		$p_{(trend)} = 0.03$
Accumulated dose*[§] (duration (y) x dose (g))								
1-499	422	0.8 (0.7-0.9)	337	0.8 (0.7-1.0)	62	0.7 (0.5-1.0)	23	1.2 (0.6-2.2)
500-999	178	0.6 (0.5-0.7)	146	0.6 (0.5-0.7)	29	0.6 (0.4-0.9)	3	0.2 (0.04-0.7)
>999	53	0.4 (0.3-0.5)	46	0.4 (0.3-0.6)	6	0.3 (0.1-0.7)	1	0.2 (0.03-1.6)
		$p_{(trend)} = 0.15$		$p_{(trend)} = 0.25$		$p_{(trend)} = 0.34$		$p_{(trend)} = 0.84$
Recency of smoking cessation								
1-10	146	0.8 (0.7-1.0)	114	0.8 (0.6-0.9)	27	1.0 (0.7-1.6)	5	0.7 (0.3-1.9)
>10	85	0.9 (0.7-1.1)	64	0.8 (0.6-1.0)	13	1.1 (0.6-2.1)	8	1.8 (0.7-4.2)
		$p_{(trend)} = 0.55$		$p_{(trend)} = 0.64$		$p_{(trend)} = 0.13$		$p_{(trend)} = 0.07$

[¶] Estimates adjusted for age, BMI, birth cohort, occupational sunlight exposure.
[†] Snuff users excluded.
* Combined cigarette (one cigarette = 1 gram), cigar (one cigar = 6 grams) and pipe dose.
[§] Discrepancy in number of cases is due to missing information on dose and duration.

Table 11. Adjusted incidence rate ratios (RR)[¶] and 95% confidence intervals (CI) of all malignant melanoma combined, cutaneous malignant melanoma (CMM), cutaneous melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM) in relation to exclusive use of cigarettes, cigars, pipe, and snuff and mixed use.

	All Melanoma combined		**CMM**		**MIS**		**IMM**	
	No. of cases	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**	**No. of cases**	**RR (95% CI)**
Never tobacco users	599	1-Ref	476	1-Ref	103	1-Ref	20	1-Ref
Pure cigarette smokers	440	0.7 (0.6-0.8)	352	0.7 (0.6-0.8)	69	0.7 (0.5-0.9)	19	0.9 (0.5-1.6)
Dose (g) of cigarette tobacco smoked/day[§]								
1-9	202	0.7 (0.6-0.9)	161	0.7 (0.6-0.9)	32	0.7 (0.4-1.0)	9	0.9 (0.4-2.0)
10-19	140	0.6 (0.5-0.8)	111	0.6 (0.5-0.8)	21	0.6 (0.3-0.9)	8	1.2 (0.5-2.7)
≥20	90	0.7 (0.6-0.9)	73	0.7 (0.5-0.9)	15	0.9 (0.5-1.7)	2	0.5 (0.1-2.0)
		$p_{(trend)}$ <0.01		$p_{(trend)}$ <0.01		$p_{(trend)}$ = 0.10		$p_{(trend)}$ = 0.79
Pure pipe smokers	88	0.6 (0.5-0.7)	72	0.6 (0.5-0.8)	11	0.4 (0.2-0.7)	5	0.6 (0.2-1.7)
Pure cigar smokers	15	0.8 (0.5-1.4)	15	1.0 (0.5-1.7)	-	-	-	-
Pure snuff dippers	96	0.6 (0.5-0.8)	74	0.6 (0.5-0.8)	17	0.6 (0.4-1.1)	5	1.1 (0.4-3.1)
Duration (y) of snuff use								
1-29	85	0.7 (0.5-0.9)	66	0.7 (0.5-0.9)	16	0.7 (0.4-1.2)	3	1.2 (0.3-4.1)
≥30	11	0.5 (0.3-1.0)	8	0.5 (0.2-1.0)	1	0.4 (0.05-2.9)	2	1.0 (0.2-4.8)
		$p_{(trend)}$ <0.01		$p_{(trend)}$ <0.01		$p_{(trend)}$ = 0.08		$p_{(trend)}$ = 0.75
Mixed tobacco use*	401	0.7 (0.6-0.8)	320	0.7 (0.6-0.8)	67	0.8 (0.5-1.0)	14	0.6 (0.3-1.2)

[¶] Estimates adjusted for age, BMI, birth cohort, occupational sunlight exposure.

* Cigarette smoking and snuff use was the most common combination.

[§] Discrepancy in number of cases is due to missing information on dose.

9.3 STUDY III

Selected characteristics of the matched 207 CSCC cases and 187 controls are presented in **Table 12**. The median age at first transplantation (51 years) and the median follow-up time (6.6 years) was similarly distributed in cases and controls. Eighty-four percent had a single transplantation and the majority (95%) received a kidney graft. Study participants who developed CSCC during the first eight years post-transplantation were older at transplantation than participants who developed CSCC after eight years or more (median age 56 years and 41 years, respectively).

Table 12. Basic characteristics of cutaneous squamous cell carcinoma (CSCC) cases and controls*.

	No. of cases (%)	**No. of controls (%)**	**p[§]**
	207 (52)	189 (48)	
Age of recipient at transplantation			
0-19	4 (2)	4 (2)	
20-39	48 (23)	45 (24)	
40-59	109 (53)	103 (55)	
≥60	46 (22)	37 (20)	0.94
Median (Range)	51 (11-71)	51 (14-72)	
Time of follow-up (years)			
Median (Range)	6.7 (0.7-21.3)	6.5 (0.2-21.3)	
Calendar time of first transplantation			
1971-1980	54 (26)	38 (20)	
1981-1990	116 (56)	114 (60)	
1990-1995	37 (18)	37 (20)	0.37

* Controls were matched to cases by age (±5 years) and calendar period (± 5 years) of transplantation.

§ P-values refer to χ^2 tests to study the differences in distribution of characteristics between cases and controls.

Table 13 and **14** present crude RRs of CSCC in relation to transplant characteristics and immunosuppressive treatment. All patients were treated with Cs in combination with either Aza or CsA or in triple treatment combination (Aza+CsA+Cs), only about one percent of the patients were treated with either of tacrolimus, mycophenolat mofetil or sirolimus. Ever use of Aza and triple treatment were both associated with a statistically significant 5-fold increased risk of CSCC, compared to never use of Aza and treatment with CsA+Cs, respectively. Similarly, a high accumulated dose combination of Aza+CsA+Cs was associated with a 4.6-fold increase in risk compared to a low accumulated dose combination (weighted dose score 8-9 versus 2-3). There was no association between ever treatment with or accumulated dose of ATG+ALG or OKT-3 and risk of CSCC. Male recipients had a borderline-significant increased risk of CSCC (p=0.06).

Table 13. Crude relative risks* (RRs) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to sex of the recipient, HLA-B mismatches in the first transplantation, total number of acute rejection episodes, and post-transplant immunosuppressive treatment.

	No. of cases (%)	**No. of controls (%)**	**RR (95% CI)**	**p[§]**
Sex of recipient				
Female	56 (27)	70 (37)	1-Ref	
Male	151 (73)	119 (63)	1.6 (1.0-2.4)	0.06
HLA-B mismatches				
0	27 (15)	37 (23)	1-Ref	
1	96 (54)	74 (45)	1.9 (1.0-3.7)	
2	55 (31)	53 (32)	1.5 (0.7-3.1)	0.16
Acute rejection episodes				
0	70 (34)	72 (38)	1-Ref	
1-2	96 (46)	95 (50)	1.0 (0.6-1.6)	
≥3	41 (20)	22 (12)	1.9 (1.0-3.6)	0.11
ATG + ALG treatment				
Never	151 (73)	145 (77)	1-Ref	
Ever	56 (27)	44 (23)	1.3 (0.8-2.2)	0.22
Aza treatment				
Never	9 (4)	29 (15)	1-Ref	
Ever	198 (96)	160 (85)	5.2 (2.0-13.6)	<0.01
CsA treatment				
Never	48 (23)	40 (21)	1-Ref	
Ever	159 (77)	149 (79)	1.0 (0.5-1.8)	0.94
Immunosuppressive regimen				
CsA + Cs	9 (4)	28 (15)	1-Ref	
Aza + Cs	48 (23)	39 (21)	4.1 (1.4-12.2)	
Aza + CsA + Cs	150 (72)	121 (64)	5.3 (2.0-14.4)	<0.01
Weighted dose combinations of Aza + CsA + Cs[†]				
≤3	27 (18)	33 (27)	1-Ref	
4-5	47 (31)	42 (34)	1.5 (0.6-3.8)	
6-7	45 (30)	32 (26)	3.0 (0.9-10.2)	
8-9	31 (21)	15 (12)	4.6 (1.1-19.9)	0.20

Aza=azathioprine, CsA=cyclosporine, Cs=corticosteroids. ATG=anti-thymocyte globulin.
* Relative risks estimated by odds ratios and 95% confidence intervals (CI) using univariate conditional logistic regression models.
Controls were matched to cases by age (±5 years) and calendar period of transplantation (± 5 years).
[†] Restricted to persons who received all three drugs. The weighted dose combination is the sum of the scored accumulated dose categories of each drug, range 2(low) - 9(high).
[§] Overall statistical significance (p-value) estimated with likelihood ratio tests.

One mismatched HLA-B antigen and more than two rejection episodes increased the risk of CSCC almost 2-fold, but there was no significant trend with increasing number of mismatches or rejections.

Treatment with Aza was associated with an increased risk of CSCC during almost all time periods analyzed, and the risk was enhanced by higher dose (**Table 14**). After the entire follow-up period, the risk of CSCC in relation to accumulated dose of Aza was close to 3-fold increased in the low dose group, and more than 6-fold increased in the intermediate and high dose groups. The risk of CSCC also increased with longer treatment duration with Aza. In comparison with patients never treated with Aza, a high accumulated dose treatment up to one year conferred a RR of 5.1 (95% CI, 1.9-14.0), after five years treatment duration this risk rose to 6.9 (95% CI, 2.5-19.1). Similarly, a high mean dose of Aza during actual treatment periods increased the risk 5.7-fold compared to never treatment. Patients that after the entire follow-up period had received a high accumulated dose of Cs were at a more than 4-fold increased risk of CSCC, compared to the lowest dose group of Cs (reference category). Increasing dose of Cs was associated with an increased risk of CSCC but the low and intermediate dose groups rarely reached statistical significance. A high mean dose of Cs during all treatment periods almost doubled the risk of CSCC. Accumulated and mean dose of CsA were consistently unrelated to CSCC risk in the crude analyses.

After multivariate adjustment, the effect of one mismatched HLA-B antigen and more than two rejection episodes was reduced. While the estimated association with sex of the recipient, immunosuppressive treatment regimen, weighted dose combination of Aza+CsA+Cs, and ever use of ATG+ALG remained essentially unchanged, the association with ever treatment with Aza and CsA were more pronounced in the multivariate analyses (data not shown).

Adjusted RRs and 95% CIs of CSCC in relation to accumulated and mean dose of Aza, CsA and Cs are presented in **Table 15**. Risk estimates of CSCC in relation to accumulated and mean dose of Cs were generally unchanged. A high accumulated dose of Cs increased the risk of CSCC 2.5-fold (95% CI 1.0-6.1) after three years and 3.9-fold (95% CI 1.2-12.3) after the entire follow-up period, compared to the lowest dose category of Cs (reference category).

Risk of CSCC in association with Aza treatment was more pronounced in the multivariate analyses (**Table 15**). Patients with a high accumulated dose of Aza were at a 5.4-fold increased risk of CSCC after one year of follow-up, and after five years the risk had risen to 8.9-fold increased, compared to never treatment with Aza (**Table 15**, **Figure 9**). A significant trend in risk of CSCC with increasing dose of Aza was present for all time periods analyzed. CsA remained unassociated with risk of CSCC at one, three and five years. However, the adjusted risk of CSCC after the entire follow-up period was non-significantly almost two times increased in patients with a low and a high accumulated dose of CsA, compared to never treatment. There was no trend in risk of CSCC with increasing dose of CsA. No effect modification between Aza, CsA, Cs, age and sex could be detected in this study, however, interaction analyses were generally low-powered and were therefore difficult to evaluate.

Table 14. Crude relative risks* (RRs) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to accumulated dose (after one, three and five years, and after the entire follow-up period) and mean dose (during treatment periods and during the second and third year) of azathioprine (Aza), cyclosporine (CsA) and corticosteroids (Cs) post-transplantation.

	Azathioprine treatment			**Cyclosporine treatment**			**Corticosteroid treatment**		
	No. of cases/ controls	**RR (95% CI)**	**p[§]**	**No. of cases/ controls**	**RR (95% CI)**	**p[§]**	**No. of cases/ controls**	**RR (95% CI)**	**p[§]**
Accumulated dose									
Reference[¶]	9 / 29	1-Ref		48 / 40	1-Ref		36 / 47	1-Ref	
- Entire follow-up									
Low	41 / 54	2.9 (1.0-8.5)		50 / 47	1.0 (0.5-2.2)		52 / 47	1.7 (0.8-3.5)	
Intermediate	80 / 52	6.3 (2.2-17.7)		54 / 52	0.9 (0.4-1.9)		48 / 47	2.1 (0.9-5.2)	
High	77 / 54	6.6 (2.3-19.2)	<0.01	55 / 50	1.0 (0.5-1.9)	0.97	71 / 48	4.4 (1.6-12.2)	0.03
- 0-1 year									
Low	50 / 51	4.1 (1.4-11.9)		41 / 44	0.7 (0.3-1.7)		46 / 45	1.7 (0.8-3.9)	
Intermediate	67 / 53	5.1 (1.8-14.5)		53 / 44	0.9 (0.4-2.3)		55 / 47	1.8 (0.8-4.0)	
High	72 / 51	5.1 (1.9-14.0)	<0.01	33 / 44	0.7 (0.3-1.5)	0.56	70 / 48	2.4 (1.1-5.2)	0.18
- 0-3 years									
Low	48 / 53	3.2 (1.1-9.2)		35 / 44	0.6 (0.2-1.4)		38 / 47	1.4 (0.6-3.0)	
Intermediate	53 / 53	4.2 (1.5-12.2)		57 / 46	0.8 (0.3-2.0)		63 / 47	2.2 (1.0-5.0)	
High	92 / 53	6.3 (2.3-17.2)	<0.01	40 / 46	0.8 (0.3-1.8)	0.60	70 / 48	2.6 (1.2-5.9)	0.05
- 0-5 years									
Low	45 / 53	3.0 (1.0-8.7)		39 / 45	0.7 (0.3-1.7)		44 / 47	1.6 (0.8-3.8)	
Intermediate	56 / 53	3.9 (1.4-11.4)		47 / 48	0.6 (0.3-1.5)		48 / 47	1.9 (0.8-4.2)	
High	94 / 54	6.9 (2.5-19.1)	<0.01	50 / 47	0.9 (0.4-2.0)	0.62	79 / 48	3.2 (1.4-7.2)	0.03
Mean dose									
Reference[¶]	9 / 29	1-Ref		48 / 40	1-Ref		43 / 48	1-Ref	
- During treatment†									
Low	53 / 53	3.9 (1.4-11.2)		45 / 49	0.8 (0.4-1.7)		34 / 46	0.9 (0.4-1.6)	
Intermediate	71 / 54	5.6 (2.0-15.5)		58 / 50	1.1 (0.5-2.2)		66 / 47	1.9 (1.0-3.5)	
High	74 / 53	5.7 (2.1-15.8)	<0.01	56 / 50	1.0 (0.5-2.1)	0.83	64 / 48	1.9 (1.0-3.5)	0.02

* Relative risks estimated by odds ratios and 95% confidence intervals using univariate conditional logistic regression models.

[¶] Reference category = never use of Aza and CsA and lowest dose category of Cs.

[†] Excluding intravenously administered drugs and periods of no treatment.

[§] Overall statistical significance (p-value) estimated with likelihood ratio tests.

Table 15. Adjusted relative risks*‡ (RRs) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to accumulated dose (after six months, one, three, five years and after the entire follow-up period) and mean dose (during treatment periods and during the second and third year) of azathioprine (Aza), cyclosporine (CsA) and corticosteroids (Cs) post-transplantation.

	Azathioprine treatment		**Cyclosporine treatment**		**Corticosteroid treatment**	
	RR (95% CI)	**p§**	**RR (95% CI)**	**p§**	**RR (95% CI)**	**p§**
Accumulated dose						
Reference¶	1-Ref		1-Ref		1-Ref	
- Entire follow-up						
Low	3.1 (1.0-9.7)		1.9 (0.8-4.7)		1.9 (0.8-4.3)	
Intermediate	8.2 (2.7-25.1)	<0.01	1.4 (0.6-3.4)	0.35	1.9 (0.7-5.2)	0.09
High	8.8 (2.6-29.9)	<0.01 ξ	1.9 (0.8-4.5)	0.31 ξ	3.9 (1.2-12.3)	0.03 ξ
- 0-1 year						
Low	4.5 (1.4-14.5)		1.1 (0.4-3.0)		2.1 (0.8-5.4)	
Intermediate	6.0 (1.9-18.8)	<0.01	1.3 (0.4-4.0)	0.77	1.7 (0.7-4.2)	0.25
High	5.4 (1.8-16.2)	0.04 ξ	0.9 (0.3-2.6)	0.92 ξ	2.3 (0.9-5.6)	0.14 ξ
- 0-3 years						
Low	3.6 (1.2-11.1)		0.9 (0.3-2.7)		1.5 (0.6-3.7)	
Intermediate	5.0 (1.6-16.0)	<0.01	1.5 (0.5-4.6)	0.50	2.1 (0.9-5.2)	0.18
High	7.5 (2.4-22.9)	<0.01 ξ	1.1 (0.4-3.1)	0.84 ξ	2.5 (1.0-6.1)	0.05 ξ
- 0-5 years						
Low	3.4 (1.1-10.6)		1.2 (0.4-3.3)		1.8 (0.8-4.1)	
Intermediate	5.4 (1.7-17.0)	<0.01	0.9 (0.3-2.5)	0.78	2.0 (0.8-4.8)	0.17
High	8.9 (2.9-27.8)	<0.01 ξ	1.2 (0.4-3.3)	0.91 ξ	2.8 (1.1-7.2)	0.04 ξ
Mean dose						
- During treatment†						
Low	4.1 (1.3-12.8)		1.6 (0.7-3.9)		0.9 (0.4-1.8)	
Intermediate	6.6 (2.1-20.9)	<0.01	2.2 (0.9-5.2)	0.35	1.5 (0.8-3.1)	0.21
High	6.4 (2.1-19.6)	<0.01 ξ	1.7 (0.8-3.9)	0.24 ξ	1.7 (0.9-3.4)	0.08 ξ

¶ Reference category = never use of Aza and CsA and lowest dose category of Cs.

* Relative risks estimated by odds ratios and 95% confidence intervals using multivariate conditional logistic regression models.

‡ Estimates adjusted for sex of the recipient, and total accumulated dose of Aza, CsA and Cs.

† Excluding intravenously administered drugs and periods of no treatment with the drug.

§ Overall statistical significance (p-value) estimated with likelihood ratio tests.

ξ P-value for linear trend in risk of CSCC.

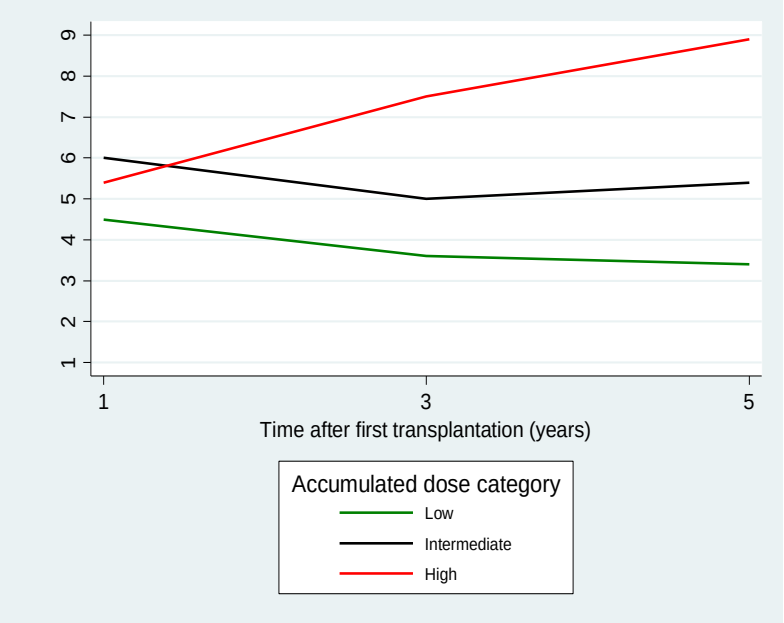

Figure 9. Adjusted relative risks of cutaneous squamous cell carcinoma (CSCC) in relation to accumulated dose of azathioprine (Aza) one, three and five years after the first organ transplantation.

9.4 STUDY IV

Characteristics of cases and controls are presented in **Table 12**. Type of organ transplanted, number of transplantations, cause of organ failure, or donor characteristics were not related to risk of CSCC (data not shown). Female donor sex and donor age over 60 years were related to an about 2-fold non-significantly increased risk of CSCC in the multivariate analyses, however, these RR were quite affected by different scenarios assumed for the missing information (sensitivity analyses).

Load, type, and combinations of infections in relation to risk of CSCC are displayed in **Table 16**. There were no statistically significant associations between any infectious parameter and CSCC risk. Compared to having no bacterial infections, a history of more than six bacterial infections in the post-transplant period was associated with CSCC by a RR of 1.5 (95% CI, 0.8-3.0), in the crude analysis. Upon adjustment for immunosuppressive drug use and sex of the recipient, this risk was slightly more pronounced (RR 1.9; 95% CI, 0.8-4.2). Herpes group virus infections, infections overall or infections with the individuals herpes viruses, CMV, HSV, VZV, and EBV, were not associated with risk of CSCC. Similarly, there was no relation between CSCC risk and hepatitis or fungal infections.

Table 16. Crude and adjusted relative risks* (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to bacterial, viral and fungal infections, infection combinations, and number of infections.

	No. of cases (%)	No. of controls (%)	Crude RR (95% CI)	Adjusted RR‡‡ (95% CI)	p§
No. of infections					
0	21 (10)	22 (12)	1-Ref	1-Ref	
1-3	73 (36)	63 (34)	1.3 (0.6-2.7)	1.5 (0.6-3.4)	
3-7	53 (26)	57 (30)	1.1 (0.5-2.3)	1.3 (0.6-3.1)	0.81
>7	56 (28)	45 (24)	1.3 (0.6-2.7)	1.5 (0.6-3.6)	0.63¶
Infection combinations					
No infections	26 (13)	31 (16)	1-Ref	1-Ref	
Only bacterial	63 (30)	52 (28)	1.4 (0.7-2.7)	1.7 (0.8-3.5)	0.16
Only viral	5 (2)	6 (3)	0.8 (0.2-3.3)	0.8 (0.2-4.0)	0.79
Bacterial/viral	53 (26)	49 (26)	1.3 (0.6-2.5)	1.6 (0.8-3.6)	0.22
Bacterial/fungal	19 (9)	24 (13)	0.9 (0.4-1.9)	1.0 (0.4-2.5)	0.93
Bacterial/viral/fungal	41 (20)	27 (14)	1.7 (0.8-3.4)	1.6 (0.7-3.7)	0.28
Bacterial infections					
0	31 (15)	37 (20)	1-Ref	1-Ref	
1-2	63 (30)	57 (30)	1.3 (0.7-2.4)	1.3 (0.7-2.7)	
3-6	61 (29)	56 (30)	1.3 (0.7-2.4)	1.7 (0.9-3.4)	0.35
>6	52 (25)	39 (21)	1.5 (0.8-3.0)	1.9 (0.8-4.2)	0.19¶
Herpes group virus infections					
Never	103 (50)	101 (54)	1-Ref	1-Ref	
Ever	102 (50)	86 (46)	1.3 (0.8-2.0)	1.1 (0.7-1.8)	0.66
CMV infection					
Never	137 (67)	133 (71)	1-Ref	1-Ref	
Ever	66 (33)	54 (29)	1.2 (0.7-1.9)	1.1 (0.7-1.9)	0.70
HSV infection					
Never	164 (79)	155 (82)	1-Ref	1-Ref	
Ever	43 (21)	34 (18)	1.3 (0.8-2.2)	1.2 (0.7-2.1)	0.59
VZV infection					
Never	180 (87)	169 (89)	1-Ref	1-Ref	
Ever	27 (13)	20 (11)	1.0 (0.5-1.9)	0.9 (0.4-1.8)	0.72
Hepatitis infection					
Never	192 (95)	173 (93)	1-Ref	1-Ref	
Ever	10 (5)	14 (7)	0.7 (0.2-1.9)	1.1 (0.4-3.5)	0.85
Fungal infection					
Never	141 (69)	132 (70)	1-Ref	1-Ref	
Ever	64 (31)	56 (30)	1.0 (0.6-1.5)	0.8 (0.5-1.4)	0.47

Controls were matched to cases by age (±5 years) and calendar period of transplantation (± 5 years).
* Relative risks estimated by odds ratios and 95% confidence intervals (CI) using conditional logistic regression models.
‡‡ Estimates adjusted for sex of the recipient and total accumulated dose of Aza, CsA, and Cs.
§ Statistical significance (p-value) estimated with likelihood ratio tests and Wald tests.
¶ P-value for linear trend in risk of CSCC.

Lastly, there were no statistically significant associations with HLA type either in the crude or adjusted analyses (**Table 17**). HLA-DR1 was non-significantly related to a 60% increase in risk of CSCC. Sensitivity analyses did not essentially change the estimates relating HLA-DR types to risk of CSCC.

Table 17. Crude and adjusted relative risks* (RR) of cutaneous squamous cell carcinoma (CSCC) after organ transplantation in relation to human leukocyte antigen (HLA) types.

	No. of cases (%)	**No. of controls (%)**	**Crude RR (95% CI)**	**Adjusted RR‡‡ (95% CI) §**	**p§**
HLA-A3					
Not carrier	133 (64)	134 (71)	1-Ref	1-Ref	
Carrier	58 (28)	48 (25)	1.4 (0.8-2.4)	1.4 (0.8-2.6)	0.21
Missing (%)	*(8)*	*(4)*			
HLA-A11					
Not carrier	172 (83)	161 (85)	1-Ref	1-Ref	
Carrier	19 (9)	21 (11)	0.7 (0.3-1.5)	0.6 (0.3-1.4)	0.24
Missing (%)	*(8)*	*(4)*			
HLA-B27					
Not carrier	167 (81)	161 (85)	1-Ref	1-Ref	
Carrier	26 (13)	21 (11)	1.0 (0.5-2.0)	1.2 (0.5-2.6)	0.70
Missing (%)	*(7)*	*(4)*			
HLA-DR1					
Not carrier	124 (60)	128 (68)	1-Ref	1-Ref	
Carrier	41 (20)	32 (17)	1.3 (0.7-2.2)	1.6 (0.9-3.1)	0.14
Missing (%)	*(20)*	*(15)*			
HLA-DR7					
Not carrier	143 (69)	135 (71)	1-Ref	1-Ref	
Carrier	22 (11)	25 (13)	0.9 (0.4-1.6)	1.0 (0.4-2.1)	0.91
Missing (%)	*(20)*	*(15)*			
HLA-DR homozygosity					
Heterozygot	114 (55)	102 (54)	1-Ref	1-Ref	
Homozygot	51 (25)	58 (31)	0.8 (0.5-1.2)	0.7 (0.4-1.2)	0.20
Missing (%)	*(20)*	*(15)*			

Controls were matched to cases by age (±5 years) and calendar period of transplantation (± 5 years).
* Relative risks estimated by odds ratios and 95% confidence intervals (CI) using conditional logistic regression models.
‡‡ Estimates adjusted for sex of the recipient and total accumulated dose of Aza, CsA, and Cs.
§ Statistical significance (p-value) estimated with likelihood ratio tests.

10 METHODOLOGICAL CONSIDERATIONS

10.1 STUDY DESIGN

The "randomized controlled intervention" study design is considered the gold standard of epidemiological study designs, however, these studies are often difficult to conduct due to ethical concerns or because of limited monetary and time resources. Therefore, observational studies, such as cohort and case-control studies, are often utilised in epidemiology to study the effect of naturally randomized exposures on risk of disease.

Cohort studies are performed within a defined group of people classified according to their exposure. The cohort is followed over time, with regard to disease occurrence, and risks of disease in the exposed and in the unexposed group are compared. There are several kinds of cohort studies. In prospective cohort studies, the exposure information is collected prior to the occurrence of outcome. Reversely, in retrospective cohort studies outcome has already occurred at the point of study initiation, and exposure information is collected thereafter or has been collected previously for purposes other than research. Due to the design of prospective cohort studies, the risk of selection bias is eliminated and the risk of misclassification bias is reduced.[42, 217] Retrospective cohort studies are more sensitive to selection and misclassification bias, especially if exposure information is collected after disease occurrence (discussed below). Cohort studies can also be open or closed. In open cohort studies, the subjects are allowed to enter and exit freely during follow-up, whereas in closed cohort studies, the cohort members are fixed at base-line and remain in the study until study termination.

The cohort study design has great potential to produce valid results. However, prospective cohort studies are often inefficient, with regard to money and time, and retrospective studies often lack detailed exposure information. To overcome these obstacles of the cohort design, a case-control study can be conducted. Case-control studies are designed to efficiently mimic the cohort study. This is achieved by selecting a control group that constitutes a representative sample of the study base from which the cases arose. Although a properly conducted case-control study (with a rare outcome) will provide comparable results to that of a cohort study in the same population,[213] there are some methodological pitfalls in the design of case-control studies that can pose a threat to the validity. For one, the retrospective nature of these studies makes them susceptible to misclassification bias (discussed below). The big challenge of case-control studies is, however, the sampling of controls. If not appropriately done, selection bias can be inherent in the study and produce invalid results (discussed below). In this aspect, the nested case-control design is methodologically superior to other case-control designs because the study base is clearly defined.[42]

Study I and II are conducted in a large retrospective cohort with prospectively collected exposure information. The exposure information is extensive and detailed and offers a rare opportunity to efficiently study exposures in relation to cancer incidence. Swedish national registration numbers and the high quality of Swedish nationwide registers ensure an almost complete follow-up of all individuals. Hence, with this information at

hand we designed an open retrospective cohort study investigating the relation between tobacco use and BMI, on the one hand, and risk of CSCC, on the other, in Study I and the impact of those exposures on risk of CMM, MIS, and IMM in Study II. In these studies we were able to separate the effect of different tobacco products and to evaluate the effect of increasing dose and duration of tobacco use on the risk of these skin cancers, something which has rarely been done before.

By using the Swedish National Patient Register we identified all solid organ transplantations performed in Sweden 1970-1997. This population-based cohort of OTRs (n=5 931) constituted a well defined study base for Study III and IV. Since we aimed to collect highly detailed data on risk factors for post-transplant CSCC we needed a cost and time efficient study design. Therefore, we conducted a case-control study, nested within the organ transplantation cohort. We chose all cases of CSCC that occurred during follow-up and a random sample of the study base (i.e. the solid organ transplantation cohort), from which the cases arose, as the control group.

10.2 VALIDITY

The existence of a causal association between exposure and disease can only be established following a large aggregation of scientific evidence and can never be based on the results from one study alone. This derives from the possible distortion of the results by chance and systematic errors (confounding and bias). However, the likelihood that an observed association in a study is causal is increased when several of the following criteria are met: (1) minimal confounding and bias, (2) limited chance variation, (3) relatively strong association, (4) dose-response relation, (5) internal consistency in subgroups of study subjects, (6) biological plausibility, and (7) temporality in order and latency time of exposure and disease.[42] Therefore, the internal validity (the probability that the study results reflect the true association in the study population) and the generalizability (the probability that the results apply to other populations) must be evaluated in every study.

10.2.1 Bias

10.2.1.1 Misclassification bias

Some degree of incorrect measurement of exposure and/or outcome occurs in most studies and causes misclassification (or information) bias. This bias is further divided in differential and non-differential misclassification depending on whether the measurement of the exposure and the outcome is systematically different with regard to each other or not. If the misclassification is random, i.e. not systemically different in the groups to be compared, the results will be diluted and the measure of association will tend to be biased towards unity. If differential misclassification bias exists, on the other hand, the results may be biased in any direction. There are several subclasses of misclassification bias such as recall, observer/interviewer, and detection bias.

Exposure misclassification bias

All studies included in this thesis are retrospective and exposure information was recorded for purposes other than research. Therefore these studies are subjected to exposure misclassification bias. However, since the exposure data was recorded prior to occurrence of the outcome - in Study I and II in questionnaires in conjunction with the health check-ups, and in Study III and IV in the patient medical records, the misclassification will not be differential with regard to the outcome. To further diminish the risk of differential misclassification bias in Study III and IV, data collectors were blinded to case/control status of the patients. Hence, any misclassification would be non-differential and consequently bias the risk estimates towards unity. In Study II and III, the observed associations would then be underestimated and not change the conclusions of the studies. In Study I and IV, a non-differential misclassification bias could potentially conceal a true relationship. In Study IV, we believe that we have captured almost all infections that occurred during the study period (1970-1997) since these patients were almost exclusively treated by certain specialized physicians (and did not receive care at other clinics) during this time (personal communication, G. Tufveson). However, it can not be ruled out that there is some misclassification in the infectious status of the patients that might dilute the effect of infections on risk of CSCC.

HLA typing method differed between laboratories, time periods and HLA loci, and included serological or genomic typing. We had no information on typing method used. Since serological typing has lower sensitivity and specificity, typing errors are likely to exist in our data set. This non-differential misclassification could potentially conceal a true relation between HLA types and CSCC in Study III and IV.

The baseline exposure information in Study I and II is judged to be valid due to the questionnaire quality control by nurses and the high consistency in reported smoking status between visits (described in *Subjects and methods*). However, time-varying exposures are measured in our studies and these relationships tend to be complicated, with intermittent pattern, or initiation/cessation of exposure. In Study I and II, information was only used from the first visit since the probability of seeking care repeatedly may be associated with the exposure or the outcome, and could therefore introduce differential misclassification. Some workers will, however, have changed exposure pattern after the first visit to the clinics, which will cause non-differential exposure misclassification. However, since the mean age at entry was 34 years, the probability of smoking initiation after entry should be small among non-smokers. Smoking cessation is more likely to have occurred during follow-up. With the reservation that workers with repeated visits may be a biased selection, Zendehdel *et al*., 2008,[64] assessed the proportion of never-tobacco users in our cohort of construction workers who had changed their smoking status during follow-up. It was found that 6.7% of the non-tobacco users were registered as current or former tobacco smokers in one of the later questionnaires. In the same individuals, about 14% of the previous smokers resume smoking and about 40% of the current smokers stop smoking between the first to the sixth visit. Therefore, the effect of previous and current smoking may be somewhat intertwined, but the comparison to the non-smokers should not be substantially affected.

Recall bias and under- or overestimation of weight and height are well-known problems in studies with self-reported anthropometric measures in relation to risk of diseases.[218] However, since height and weight were measured by nurses in Study I and II, misclassification of BMI at entry into the cohort should be minimized. In the sample of workers with repeated measures (described above), 70-80% of workers with normal weight, overweight, and obesity (BMI categories defined in *Background*) remain in these BMI categories throughout follow-up (**Table 18**). However, 80% of the underweight individuals become normal weight and 30% and 20% of the normal weight and obese individuals, respectively, become overweight. This will probably dilute the associations between BMI and CSCC, CMM, MIS, and IMM slightly.

Table 18. Change in Body mass index (BMI)* of workers with repeated measurements from entry into the cohort up to the fifth visit.

	Underweight throughout follow-up	**Normal weight** throughout follow-up	**Overweight** throughout follow-up	**Obesity** throughout follow-up
Underweight at entry	15%	80%	5%	-
Normal weight at entry	-	70%	30%	-
Overweight at entry	-	10%	80%	10%
Obesity at entry	-	-	20%	80%

* Rounded off to even 5%.
Underweight: BMI<18.5 kg/m^2, Normal weight: BMI=18.5-24.9 kg/m^2, Overweight: BMI=25-30 kg/m^2, Obesity: BMI>30 kg/m^2.

In Study III and IV, detailed data on immunosuppressive drug treatment was collected for all individuals included in the study. In the occurrence of gaps in follow-up time we sought the information in other clinics or hospitals, but were sometimes unable to find it. When a dose change occurred in such gaps, the median date between the last date of the old dose and the first date of the new dose was adopted. This rarely occurred during the first year post-transplantation but increased somewhat in frequency thereafter. Three years or more after the transplantation, about five percent of the patients had such gaps that encompassed three to twelve months. Moreover, although we had information on the prescribed drugs, doses and treatment durations, we had no information on the adherence to the treatment or the individual bioavailability of the drugs. When we found information that patients had deviated from the treatment plan, we used the most correct information available. If there was no such information, we collected the data according to intention-to-treat. Non-adherence with the treatment is a known problem in OTRs.[219] Depending on the definition used for non-adherence, the prevalence varies from approximately 15% to 30%.[219] However, the largest part of non-adherence in renal transplant recipients is of the accidental type and occurs relatively infrequently.[219] Therefore, we do not expect misclassification of immunosuppressive drug doses from neither non-adherence, nor from gaps in follow-up time to affect our results substantially.

Outcome misclassification bias

Recorded NMSC in the Swedish Cancer Register, ICD-7 191.1-191.9, comprises all skin malignancies apart from CMM. However, since registration of BCC did not start until 2003, CSCC constitutes 92.1% of all cases registered with NMSC, another 6.7% are CSCC/BCC mixed, and only 1.2% are other types of skin malignancies.[18] Although MIS are to be reported to the Cancer Register, the completeness of this data has not been evaluated. Likewise, due to the increasingly more common eye-sparing treatment of IMM, the completeness in the reporting of these cases to the Cancer Register is uncertain. In a previous study by Bergman *et al.*, 2002,[7] 140 additional cases were found by a search in the archives of the two hospitals in Sweden that offered eye-sparing treatment during the study period. These constituted about 5% of all cases. Apart from missed cases there might also be false cases of IMM since this disease is diagnosed clinically. In conclusion, we believe we have captured virtually all cases of CSCC and CMM in the studies included in this thesis, but some cases of MIS and IMM might have been missed or erroneously included. However, non-registration or misdiagnosis of MIS and IMM should not be associated with the exposure and would therefore produce non-differential misclassification.

10.2.1.2 Selection bias

Case-control studies are particularly susceptible to selection bias but this phenomenon may also occur in retrospective cohort studies. In case-control studies, selection bias arises when the probability of selecting a case or a control is associated with the exposure under study. In retrospective cohort studies, selection bias occurs either when disease status differentially influences the selection of exposed and unexposed subjects, or when loss to follow-up is differential with regard to both exposure and outcome.

Selection bias is unlikely to have occurred in the studies included in this thesis. In Study I and II, the cohort was defined prior to the occurrence of the outcome and hence, disease status did not influence the selection of the subjects. Furthermore, due to the high quality of the Swedish registries, we had virtually complete follow-up of all subjects and the losses to follow-up are unlikely to have been associated with both the exposure and the outcome. In Study III and IV, all cases that arose during follow-up were included, and the controls were randomly chosen from the study base from which the cases arose. Therefore, the exposure status did not influence the selection of cases and controls in these studies. However, a selection bias was introduced in Study III and IV when the controls were matched to the cases, but this bias is controlled by using conditional logistic regression in the analyses as discussed in *Statistical analyses*.

Selection bias may also be introduced by non-participation, if the probability of participating is associated with the exposure and with the outcome. In Study III and IV, living subjects were asked for informed consent to collect information from their medical records. The participation rate was high - less than two percent refused participation and only four percent never replied to the letter, reducing the risk of selection bias. Furthermore, 53 patients (12%) were lost to follow-up since their medical records could not be located. However, these losses constituted both cases (n=23) and controls (n=30) and the exposure should not be associated with the probability of finding the medical records.

Study I and II were conducted within an occupational cohort. A cohort consisting of working individuals are commonly healthier than the general population. This is referred to as the healthy worker effect and is a kind of selection bias. Hence, our cohort was probably healthier than the general population at entry into the cohort. This might influence the generalizability of our results but it should not affect the association between BMI, tobacco use and the risk of CSCC, CMM, MIS and IMM.

10.2.2 Confounding

Confounding is the distortion of the exposure-disease association induced by another risk factor for the outcome (in both the exposed and unexposed) that also affects the exposure (**Figure 10**). Furthermore, a confounder cannot be an intermediate factor in the causal chain between the exposure and the disease. Confounding may exist in all epidemiological study designs and can be approached in various ways in the different phases of the study. In the design phase, confounding can be eliminated or reduced by randomization, restriction (e.g. the subjects in Study I and II were restricted to male workers) or by matching (e.g. in Study III and IV controls were matched to cases by age and calendar period of transplantation). In the analysis phase, confounding can be managed by stratification or multivariate adjustment in regression models. Which potential confounders to collect information on and adjust for is not always straightforward and usually requires prior knowledge of the disease under study and of the relationships between different exposures.

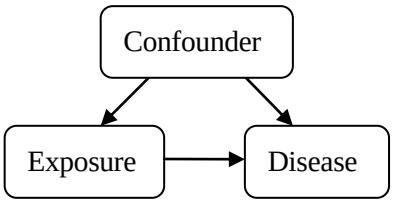

Figure 10. Graph of the relationship between exposure, disease and confounder.

In Study I and II, the study subjects were restricted to male workers and all estimates were adjusted for age. The effect of arsenic exposure on risk of CSCC was explored by using the job task coding available in this cohort. However, job tasks associated with arsenic exposure were not related to risk of CSCC and therefore the results were not adjusted for this exposure. In Study III and IV, cases and controls were matched on age and calendar period of transplantation and the results from the multivariate analyses were adjusted for sex of the recipient and for the total accumulated doses of Aza, CsA and Cs.

In this thesis, one obvious limitation is the lack of information on sunlight exposure. Sunlight exposure is a known risk factor for CSCC and CMM/MIS, and if sunlight exposure is associated with tobacco use, it may confound the relationship between tobacco use and CSCC and CMM/MIS (**Figure 11**).

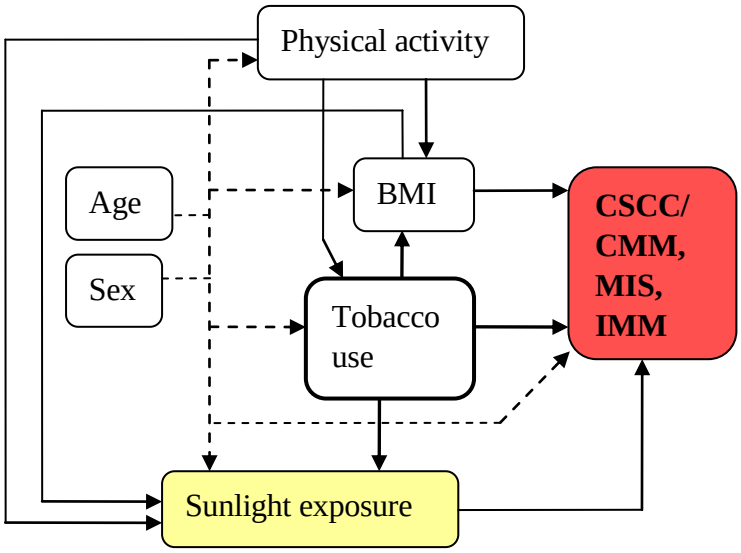

Figure 11. Graphic illustration of the relationships between the exposures studies in Study I and II, sunlight exposure, and cutaneous squamous cell carcinoma (CSCC), cutaneous malignant melanoma (CMM), malignant melanoma *in situ* (MIS), and intraocular malignant melanoma (IMM).

Previous studies have found that smoking was associated with sunbathing behavior,[220, 221] use of indoor tanning devices,[222] and less use of sun screen.[220, 223] If smoking and sunlight exposure were positively associated with each other, then confounding by sun would falsely overestimate an increased risk and underestimate a decreased risk of CSCC and CMM/MIS in smokers. However, during the study period of Study I and II, the hazardous effects of sunlight exposure were largely unknown and tanning might have been part of a healthy lifestyle in non-smokers. Furthermore, a high physical activity level is positively associated with sunlight exposure[221] and inversely associated with smoking.[224] In this scenario, non-smokers would be more exposed to sunlight than smokers and both a positive and a negative association with tobacco use would appear to be smaller than the true association.

In the cohort of Swedish construction workers, the effect of occupational sunlight exposure has previously been reported to be unrelated to risk of CSCC.[210] Furthermore, since the socioeconomic status of the workers was uniform, the leisure time and recreational exposure pattern should be relatively similar within the cohort. Although this argues against substantial confounding by sunlight exposure, the possibility of residual confounding cannot be ruled out.

In Study II, the results were adjusted for occupational sunlight exposure, estimated from job task codes, and for time trends in tanning behavior, approximated by birth cohort. Although the recreational exposure pattern should be similar within the cohort, as described above, there might still be residual confounding by sunlight exposure. Furthermore, being overweight and obese has been reported to be associated with high risk tanning behavior.[221] If this relationship exists, residual confounding by sun exposure could be a problem in Study II where a high BMI was associated with risk of CMM.

In Study III and IV, sunlight exposure should not be associated with the probability of receiving a specific immunosuppressive treatment regimen - at least not until the skin effect becomes evident at a late stage (**Figure 12**). Sunlight exposure has been shown to reactivate HSV and HPV infections.[225] However, infection with HSV was unrelated to risk of CSCC and the association is therefore unlikely to be confounded by sun. UVR from the sun also suppresses immunity systemically, although the reduced protection against bacterial, viral and fungal infections is limited.[152] Therefore, confounding by sunlight exposure is not of great concern in these studies.

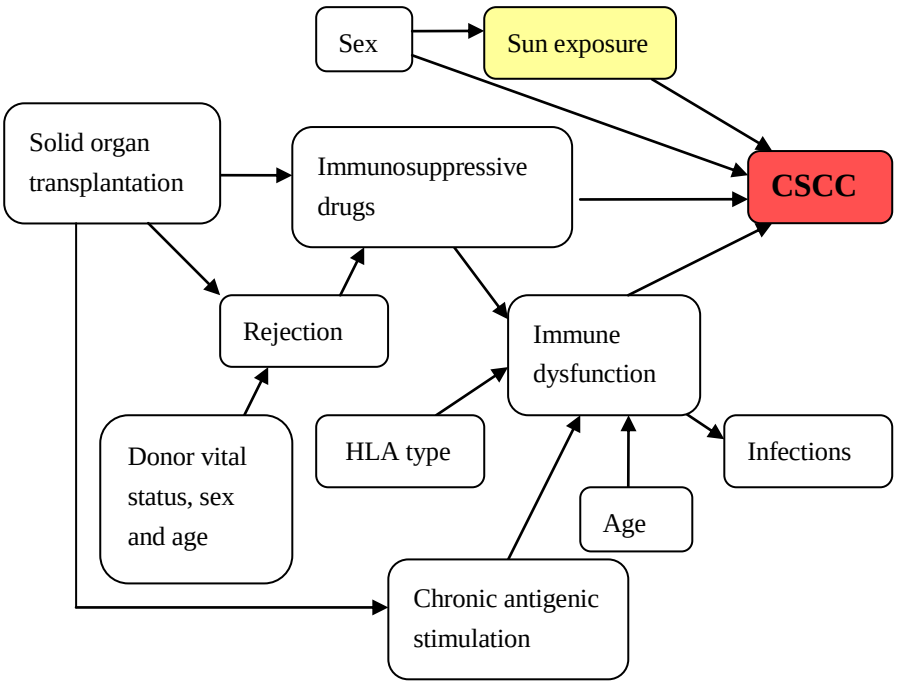

Figure 12. Graphic illustration of the relationships between exposures after solid organ transplantation, sunlight exposure, and cutaneous squamous cell carcinoma (CSCC).

10.2.3 Precision and chance

Highly unlikely events occur frequently by chance in our daily lives and this is also true for epidemiological research. The probability that chance, or random error, produced the results of a study can be evaluated statistically by p-values and confidence intervals. The significance level of the studies included in this thesis was set to 0.05. This infers that we with 95% certainty excluded the possibility that the observed association (or a more extreme association) was due to chance alone. Precision is the repeatability of the results with further measurements and is visualized in the width of the confidence interval.

P-values and confidence intervals are affected by study size and strength of the association studied. Study I and II were conducted in a large cohort and therefore the probability of chance was reduced and precision was increased. However, the power to

detect an association in the analyses of exclusive cigar smokers and in the extremes of BMI was reduced due to the low number of subjects in these exposure subgroups. In Study II we therefore grouped BMI into two categories, underweight/normal weight and overweight/obese. Random error was also difficult to exclude in the analyses of IMM due to the rarity of this outcome.

The size of Study III and IV (about 200 cases and 200 controls) is relatively large. Nevertheless, the statistical power to detect weak associations in these studies is limited. Moreover, the frequent use of some drugs (Aza) made the unexposed group small, thus reducing precision.

With multiple testing the p-values lose their collective interpretability. However, the studies included in this thesis have had a high internal consistency in subgroups, with dose-response relationships of effects, thus reducing the probability that the results were due to chance alone. Nevertheless, chance can never be entirely ruled out.

10.3 EXTERNAL VALIDITY

The external validity, or the generalizability, is the probability that the estimated association between exposure and disease in the study group will apply to other populations. In general, different populations have diverse genetic backgrounds and environmental exposures, thus restricting comparisons between studies to some extent. In Study I and II, the healthy worker effect might have affected the external validity since these individuals are not directly comparable to the general population (discussed above). Also, the socioeconomic status of the construction workers is not representative of the general population, and the results cannot be generalized to women since these studies were restricted to men. The results of Study III and IV only apply to other organ transplant populations, due to the unusual exposure pattern in these individuals, though apart from this constraint the generalizability is expected to be high.

11 DISCUSSION

11.1 STUDY I

In the large retrospective cohort of construction workers used in Study I we found no relation between cigarette, pipe or cigar smoking and risk of CSCC. This is supported by two case-control studies including 88 and 178 CSCC cases, respectively (**Table 1**, see *Background*).[50, 51] In contrast, a 50% increased risk of CSCC in current cigarette smokers compared to never smokers was found in a large prospective cohort study.[47] However, there was neither a correlation with former smokers nor a dose-response effect in this study. Smokers were also found to be at an increased risk of CSCC in two case-control studies,[48, 49] one of which also found that risk increased with higher doses.[48] Additionally, De Hertog *et al*., 2001,[48] found an increased risk of CSCC in pipe smokers but not in cigar smokers. However, these results were based on few cases. The inconsistent findings with regard to cigarette and pipe smoking could be due to (residual) confounding by sunlight exposure as discussed earlier. Either the studies (including ours) that found no association were negatively confounded by sun (increased sunlight exposure in non-smokers) or studies reporting an increased risk of CSCC in smokers were positively confounded by sun (increased sunlight exposure in smokers).

Snuff use was associated with a decreased risk of CSCC in our study. To our knowledge, no previous studies have investigated this association. Since this estimate was based on few cases, chance cannot be ruled out as an explanation of this finding.

Our results regarding BMI are consistent with the only study that has investigated the association previously.[81]

11.2 STUDY II

Study II has presented evidence of a consistently decreased risk of CMM and MIS in tobacco smokers and snuff users compared to never tobacco users. The risk was reduced by longer duration of tobacco smoking and snuff use. Likewise, increasing quantity smoked was related to a decreased risk of CMM, although the trend was not significant. A similar risk pattern associating tobacco smoking with IMM was observed, but risk estimates were based on few cases and were seldom statistically significant. Lastly, overweight/obesity (BMI $\geq$25 kg/m^2) was related to an increased risk of CMM, but not of MIS or IMM, compared to normal weight/underweight (BMI <25 kg/m^2).

Our results of a negative association between cigarette smoking and CMM risk is consistent with several previous studies (**Table 2**, see *Background*).[68-70, 72] Additional studies presented an indicated, non-significant reduced risk of CMM in cigarette smokers compared to non-smokers,[66, 67, 71, 73, 74] and some studies found no association between tobacco smoking and risk of CMM.[48, 75] Previous studies on pipe and cigar

smokers have found no association with risk of CMM.[71, 73, 75] Snuff use has only been investigated in one prior study that reported an indicated decreased risk of CMM, but the estimates were based on few cases and were therefore difficult to interpret.[76]

A possible mechanism for the observed inverse association between tobacco use and risk of CMM may be through a reduced activity of cyclooxygenase-2 (COX-2) in smokers. Smoking has been shown to affect immunity[35, 36, 39-41], cutaneous blood flow[46, 226] (see *Background*), and to reduce the erythematic reaction following sunburn.[226] These effects of smoking may partly be mediated by a decreased production of prostaglandins.[41] Prostaglandin synthesis is regulated by COX-2 and COX-2 has been found to be up-regulated in keratinocytes[227] following UVR treatment and in CMM and melanoma cell-lines.[228] In support of this hypothesis, a decreased risk of CMM with daily intake of non-steroidal anti-inflammatory drugs (COX-2 inhibitors) was observed in a case-control study conducted by Harris *et al*., 2001.[229] However, this finding was not reproduced in two large prospective cohort studies.[230, 231] The observed association may also be explained by confounding by sunlight exposure if tanning behavior was part of a healthy life-style in the non-smokers but not in the smokers (see *Methodological consideration*). Smoking would in this scenario falsely appear to be associated with a decreased risk of CMM/MIS.

Our findings with regard to overweight/obesity in men is supported by a few previous studies (**Table 3**, see *Background*),[83, 85, 86] and in some additional studies, non-significant increased risks have been reported.[66, 69, 87] Several hormonal alterations occur in overweight/obese individuals, including increased concentrations of GH, IGF-1, insulin, testosterone (women), and oestradiol, and decreased levels of testosterone in men.[78] All of these hormones may influence CMM risk by promoting cell proliferation, inhibiting apoptosis (insulin, IGF-1), affecting skin thickness, wound healing, pigmentation, and skin immunity (testosterone, oestradiol).[78, 232] Maintenance of a chronic inflammation in overweight/obese persons may also affect the risk of CMM.[80] Furthermore, since several studies have found increased risks with increasing height and BSA,[67-69, 85, 86] it has been suggested that an increased number of cells at risk might explain the findings. Additionally, BMI may be related to other risk factors for CMM such as sun exposure or socioeconomic status. Lastly, residual confounding by age could distort the association since BMI tends to increase with age.

11.3 STUDY III AND STUDY IV

In a large nested case-control study of OTRs, with detailed recording of exposure information, we have found evidence that treatment with Aza is strongly associated with risk of CSCC. We observed highly significant trends of increasing risk of CSCC with increasing accumulated and mean dose of Aza, and the risk was more pronounced with longer treatment duration. Furthermore, we observed an association between high accumulated dose of Cs after longer treatment durations (at least three years) and risk of CSCC. In contrast, CsA was not associated with risk of CSCC.

Previous studies investigating the impact of immunosuppressive drugs (Aza, CsA, Cs) on risk of CSCC have had several limitations such as crude exposure assessment and small size (discussed in *Background*). An inherent problem in the research of drug regimens that changes with time is also the use of historical controls that could potentially cause differences in comparison groups regarding inclusion criteria for organ transplantation, screening, diagnostic methods, recording of cancers, and treatment of other concurring diseases. Nevertheless, our finding with regard to Aza is supported by a few previous studies (**Table 4**). Ramsay *et al*., 2003,[56] found a 2.4-fold increased risk of CSCC in patients ever treated with Aza and Hiesse *et al*., 1997,[108] found that a treatment regimen without Aza significantly decreased risk of NMSC in OTRs. Additionally, two prospective studies, one larger cohort study and one smaller randomized controlled trial, detected an association between a high mean dose of Aza and an increased risk of skin carcinoma.[113, 114] In contrast, no relation between administered doses of Aza, CsA or Cs was observed in three small studies.[55, 115, 116]

A possible underlying mechanism of this observation is an enhanced effect of UVR on skin carcinogenesis by Aza treatment. Kelly *et al*., 1987,[233] showed that Aza has a strong inducing and promoting effect on tumors in hairless mice exposed to UVR. Furthermore, O'Donovan *et al*., 2005,[234] found that Aza photosensitizes the skin to UVR by changing the absorption interval in DNA when the metabolite 6-TG is incorporated. The absorption of UVR then causes DNA damage.

Most previous studies have not found differences in risk of CSCC with different treatment regimens,[95, 100, 107, 109, 111] though some have found an increased risk or earlier occurrence with triple treatment (Aza+CsA+Cs) compared to double treatment with Aza+Cs.[96, 108, 110] From these findings it has been hypothesized that risk of CSCC post-transplantation is conferred by a heavy immunosuppressive burden rather than by specific immunosuppressive drugs. To address this hypothesis we created a variable for increasing drug dose burden in patients receiving all three drugs of Aza, CsA and Cs. Consistent with the immunosuppressive load hypothesis, we found that a high dose load increased the risk of CSCC compared to a low dose load, but the magnitude of risk did not differ much from the risk conferred by Aza separately.

In Study IV we investigated the effect of a heavy immunosuppressive load on risk of CSCC through a different approach. The number and types of infections are known to correlate to level of immunosuppression[235] and were therefore used as a proxy for immunosuppressive load. Neither total number of overall infections nor type of infection was associated with risk of CSCC in OTRs. However, our results of possible association with a large number of bacterial infections (>6) may indicate that a generally weakened immune defense also increases the risk of CSCC.

Although there are several observations supporting the immunosuppressive load theory, such as the increased frequency of oncogenic virus-associated malignancies occurring post-transplantation[94] and the regression of tumors, such as lymphomas, upon lowering doses of immunosuppressive drugs,[236] there are some counterarguments to this theory with regard to CSCC. Firstly, even though CSCC is increased in persons infected with human immunodeficiency virus (HIV), the BCC to CSCC ratio resembles the ratio in

the general population and not the reversed ratio in the organ transplant population.[237] This indicates that there are some additional factors implicated in skin carcinogenesis in OTRs that are not present in HIV patients. Secondly, CSCC usually does not develop during the period of most intense immunosuppression, i.e. month two to six post-transplantation. Likewise, HPV infections, previously implicated in CSCC etiology, commonly do not emerge until after the first six-month period post-transplantation, and persist despite reduced immunosuppressive dose load. These observations, together with the results from Study III, indicate that the risk of CSCC is not primarily driven by the biologically achieved level of immunosuppression, but that the immunosuppressive therapy affects the risk by another mechanism.

12 CONCLUSIONS

- Tobacco smoking appears not to be associated with risk of CSCC. Snuff use may be related to a decreased risk of CSCC, but chance cannot be ruled out.

- Tobacco smoking and snuff use seem to be associated with a reduced risk of CMM and MIS. There was also an indicated reduced risk of IMM in tobacco smokers that could not be established due to low statistical power.

- BMI may be associated with an increased risk of CMM. Risks of CSCC, MIS and IMM were unrelated to BMI.

- Risk of CSCC post-transplantation does not appear to be an effect of an overall immunosuppressive drug load, but important differences in risk are likely to be conferred by specific drugs.

- Azathioprine treatment was strongly associated with risk of CSCC in organ transplant recipients. High accumulated dose during longer treatment periods of corticosteroids may increase the risk of post-transplant CSCC. Cyclosporine was unrelated to risk of CSCC in our study.

- Neither an overall infectious burden, as an approximation of immunosuppressive level, nor infections with specific microorganisms appear to be associated with risk of CSCC.

- We observed no relation between HLA type or mismatch and risk of CSCC in Swedish organ transplant recipients.

- There seems not to be an association between number of transplantations, type of organ transplanted, cause of kidney failure, and donor vital status and risk of CSCC.

13 IMPLICATIONS

Skin cancer has increased epidemically in the fair-skinned populations over the past several decades. While many epidemiological studies have investigated the etiology of CMM, research on CSCC is scarce since this cancer entity is rarely reported to cancer surveillance systems. Nonetheless, many questions regarding both CMM and CSCC remain unanswered and, although the studies included in this thesis adds to the evidence, some new hypotheses have been generated that need to be evaluated in future studies.

Tobacco smoking has previously been linked to several malignancies. The evidence regarding the association between CSCC and smoking has been inconsistent. In our study there was no association between tobacco smoking and risk of CSCC, but, in light of previous studies, the true association is still not established and claims further research. Future studies should include careful assessment of smoking habits over time and of sunlight exposure, since this exposure is potentially confounding the association between smoking and risk of CSCC. Other risk factors, such as constitutional characteristics and pharmacological treatment, should also be collected to investigate possible interactions with smoking.

The observed inverse relation between tobacco use and CMM deserves confirmation or rejection in future studies. These studies should employ a careful exposure assessment as suggested in the paragraph above. A decreased risk of CMM in smokers does not imply that smoking should be utilized for prevention, but it might provide clues to the etiology of CMM. The interaction between sunlight exposure and smoking in relation to CMM occurrence might be particularly interesting to investigate since there is some evidence of a reduced erythematic/inflammatory reaction in smokers following sunburn. Additionally, future efforts should be invested in further elucidating the inflammatory mechanisms following sunburn to increase our understanding of why intermittent intense sunlight exposure is associated with risk of CMM. In this context, genetic association studies could be employed to investigate the relation between genes regulating inflammatory responses to UVR and risk of CMM.

Overweight and obesity are potential modifiable risk factors for CMM. However, the association is not established. Additional studies should investigate the impact of overweight and obesity on risk of CMM while controlling for tanning behavior and constitutional characteristics. Also, similar to meta-analyses on anthropometric measures in women,[89] meta-analyses on anthropometric measures in men should be conducted to summarize the evidence available today. Interestingly, there is some indication of a difference between men and women in the association between BMI and CMM (Table 3). This effect may be a consequence of differences in tanning behavior in overweight men and women, but there are also possible molecular mechanisms, such as chronic inflammation or altered hormonal balance (see *Discussion*), that could cause these differences. The effect of altered sex steroid hormone levels on estrogen receptor expression and activity in CMM is an interesting question. Another future research topic is the survival rate after CMM among overweight/obese individuals. Does the

survival rate differ compared to normal weight individuals and in that case, by what mechanisms?

The occurrence of CSCC is substantially increased in organ transplant recipient but the underlying mechanisms are largely unclear. In this thesis we have presented evidence and arguments against the level of immunosuppression as the primary cause of CSCC. We found a highly increased risk of CSCC with treatment using specific immunosuppressive drugs, i.e. primarily Aza but also, to a smaller extent, Cs. These findings have several implications. Firstly, most previous studies investigating the effect of immunosuppressive drugs in relation to risk of CSCC post-transplantation have been under-powered and have used crude exposure assessments. Thus, the results with regard to Aza should be re-evaluated in future epidemiological studies with detailed assessment of treatment dose and duration. In such studies, information on sunlight exposure needs to be collected and genotyping for polymorphisms of enzymes involved in Aza metabolism should also be performed (e.g. glutathione *S*-transferase), to examine a possible interaction between Aza treatment, metabolism and UVR. Secondly, the risk of CSCC in other patient groups, for instance patients with Crohn's disease, ulcerative colitis, and rheumatic arthritis, treated with Aza is largely unknown and deserves further research since there might exist safer alternative treatments. Thirdly, the association between Aza and risk of CSCC, together with the lack of a relation to immunosuppressive load, provides clues to the etiology of CSCC after organ transplantation. Studies further separating the direct carcinogenic, and indirect immunosuppressive, effects of the immunosuppressive treatment are called for. In this context, the interaction between immunosuppression, immunosuppressive agents, HPV, HLA and UVR in the development in post-transplant CSCC deserves more attention.

Cs is widely used for many medical conditions in different administration forms, not the least in treatment of different skin conditions. For instance, in children these medications are used in the treatment of asthma and atopic dermatitis, two diseases increasing in incidence. The association between Cs and CSCC, as well as other skin malignancies, is insufficiently investigated. This association warrants further research, with careful exposure assessment of different administration forms of Cs treatment, other pharmacological treatments, and sun exposure, in prospective epidemiological studies.

14 SVENSK SAMMANFATTNING

Bakgrund: Antalet patienter med hudcancer i befolkningen har ökat snabbare än någon annan cancerform under de senaste 40-50 åren. Det är okänt om det finns något samband med vanliga exponeringar, som t.ex. tobaksbruk och övervikt.

Risken för skivepitelcancer i huden (CSCC) är kraftigt förhöjd hos organtransplanterade patienter, men vi vet inte varför. I studier av kombinationsbehandlingar med immunhämmande läkemedel har det föreslagits att risken för CSCC efter transplantation snarare beror på den totala hämningen av immunsystemet än på vissa specifika mediciner.

Syfte och metod: Det första syftet med denna avhandling var att utvärdera tobaksbruk och övervikt (BMI) i relation till risken för CSCC, malignt hudmelanom (CMM), förstadium till melanom (MIS) och ögon-melanom (IMM). I detta syfte använde vi ett stort register av svenska byggnadsarbetare (n≈340,000) med nästan komplett uppföljning.

Det andra syftet var att bestämma de enskilda effekterna av immunhämning, infektioner, immunhämmande läkemedel och andra potentiella riskfaktorer i organtransplanterade patienter på risken för CSCC. Vi genomförde därför en fall-kontrollstudie, med ursprung i ett register av organtransplanterade patienter (n≈6,000), och inhämtade detaljerad information från patientjournaler med hjälp av ett standardiserat frågeformulär.

Resultat: Tobaksrökning var associerat med en 30-50% minskad risk för CMM, MIS och IMM, jämfört med icke-tobaksanvändare. Risken för CMM och MIS minskade också med längre varaktighet och högre dos av tobaksrökning. Likaså var uteslutande användning av cigaretter, pipa eller snus relaterat till en minskad risk för CMM och MIS. Tobaksrökning var inte associerat med risken för CSCC, jämfört med icke-tobaksanvändare, men snusning var relaterat till en 40% minskad risk för CSCC.

Övervikt/fetma ökade risken för CMM med 30% jämfört med normalvikt och undervikt, men påverkade inte risken för MIS, IMM eller CSCC.

Azatioprin-medicinering (Aza) ökade risken för CSCC efter organtransplantation avsevärt i alla undersökta perioder. Dessutom ökades risken för CSCC av en hög ackumulerad dos av kortikosteroider (Cs), jämfört med en låg ackumulerad dos, efter en längre tids behandling. Ciklosporin-behandling var inte associerat med risk för CSCC efter organtransplantation. Antal och typ av infektioner var inte kopplade till risken för CSCC och det var inte heller vävnadstyp och -matchning, antal transplantationer och avstötningsreaktioner, organtyp eller karakteristika för organgivare.

Slutsats: Vi fann att tobaksbruk var associerat med en minskad risk för CMM och MIS, men inte associerat med risk för CSCC. Relationen till IMM är oklar. Likaledes var övervikt/fetma i vår studie relaterat till en ökad risk för CMM men inte för CSCC, MIS och IMM. Relationerna mellan tobaksbruk, BMI och CMM bör undersökas vidare.

Uppkomsten av CSCC efter organtransplantation verkar inte vara resultatet av den totala immunhämningen, utan vi fann att risken är olika beroende på vilket immunhämmande läkemedel som använts. Andra undersökta faktorer i samband med organtransplantation verkar inte påverka uppkomsten av CSCC. Det behövs flera studier för att vidare separera de direkta cancerframkallande, och de indirekta immunhämmande, effekterna av immunhämmande läkemedelsbehandling i organtransplanterade patienter och i andra patientgrupper.

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