Epidemiologic aspects of skin cancer in organ-transplant recipients

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ISBN: 978-90-9026065-5
Cover design and lay out by: In Zicht Grafisch Ontwerp, Arnhem
Printed by: Ipskamp Drukkers BV, Enschede

The printing of this thesis was financially supported by DDL Diagnostic Laboratory, Voorburg, Astellas Pharma B.V., Pfizer B.V., Meda Pharma B.V., LEO Pharma B.V., Galderma, Stiefel GlaxoSmithKline company, Novartis Pharma B.V.

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Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 12 april 2011
klokke 15:00 uur

door

Hermina Christina Wisgerhof
geboren te Papendrecht
in 1981

Promotiecommissie:

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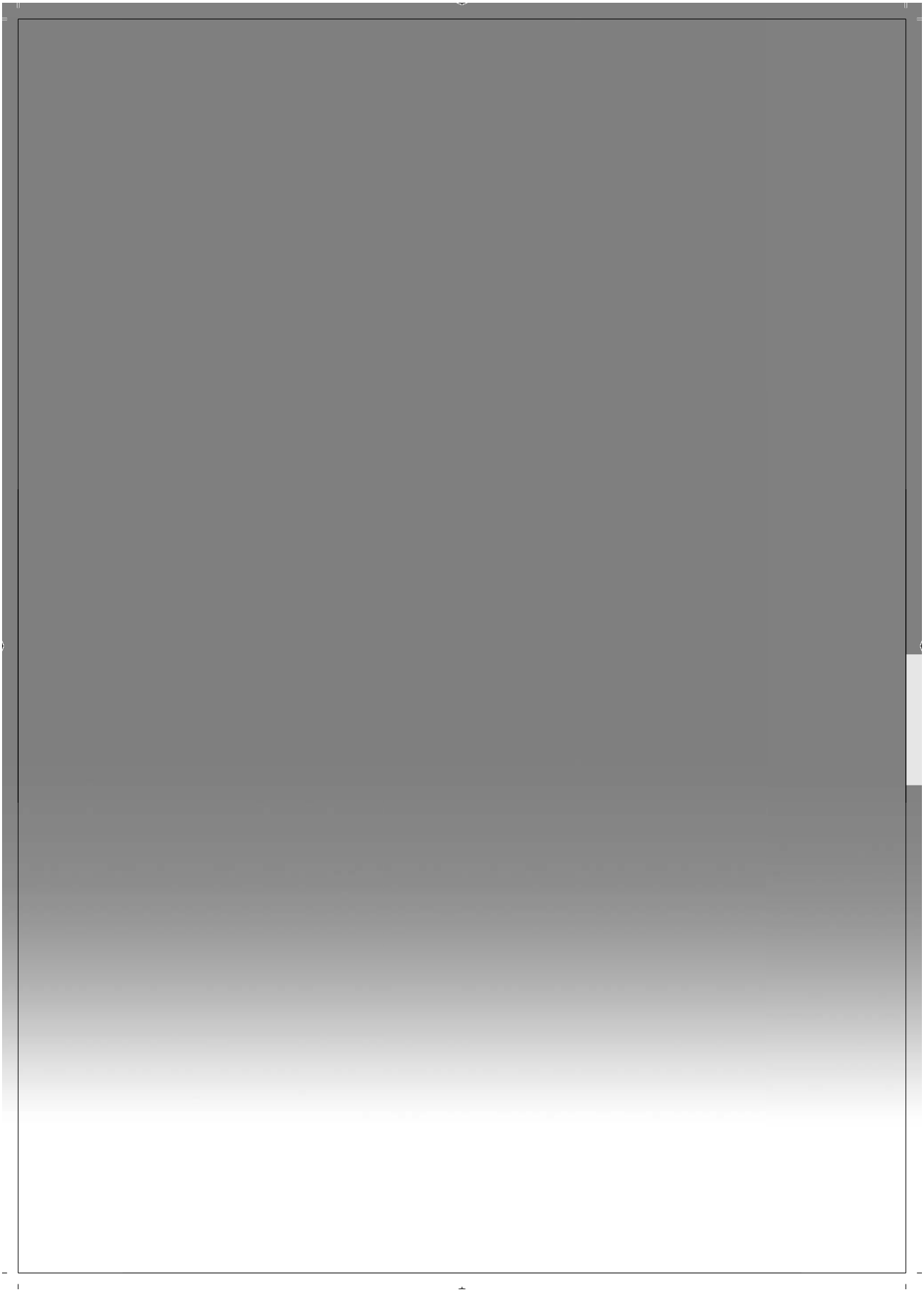
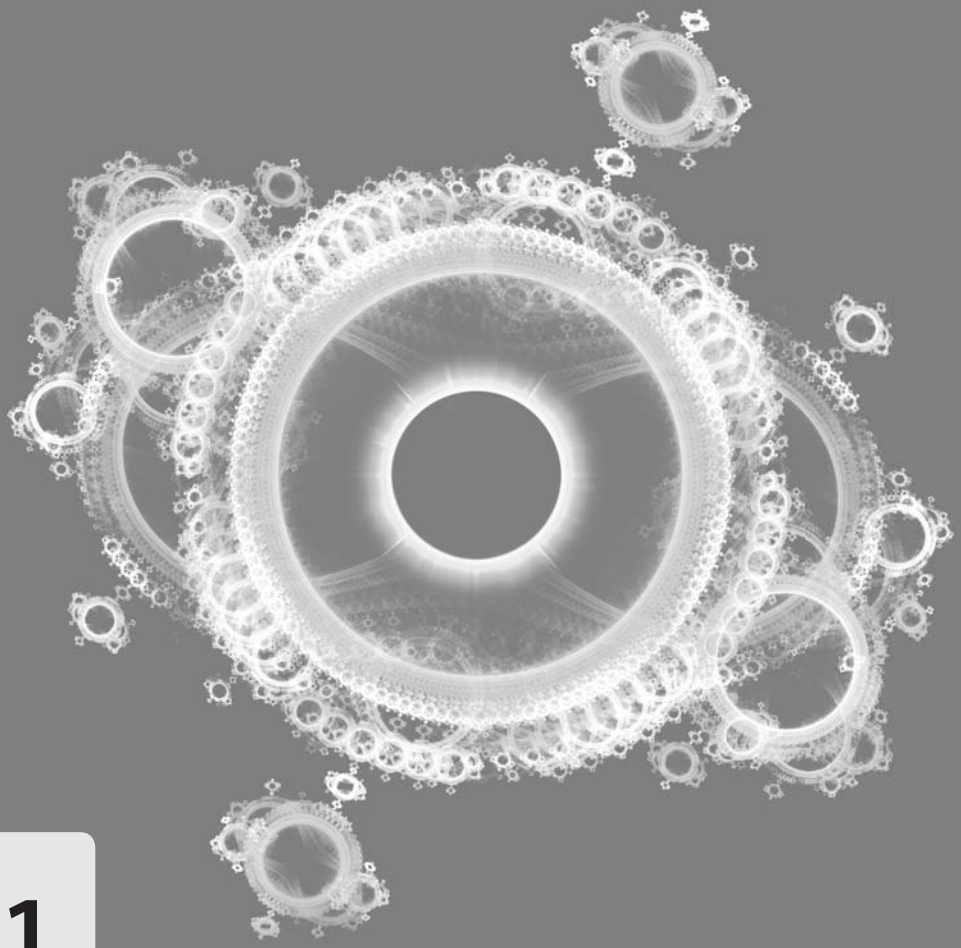




1

General introduction

Parts of this introduction have been published before as:

The epidemiology of transplant-associated keratinocyte cancers in different geographical regions. *Cancer treatment and research. 2009; 146: 75-95*

Etiological factors in cutaneous carcinogenesis - an introduction. *Cancer treatment and research. 2009; 146:97-100*

Organ transplantation

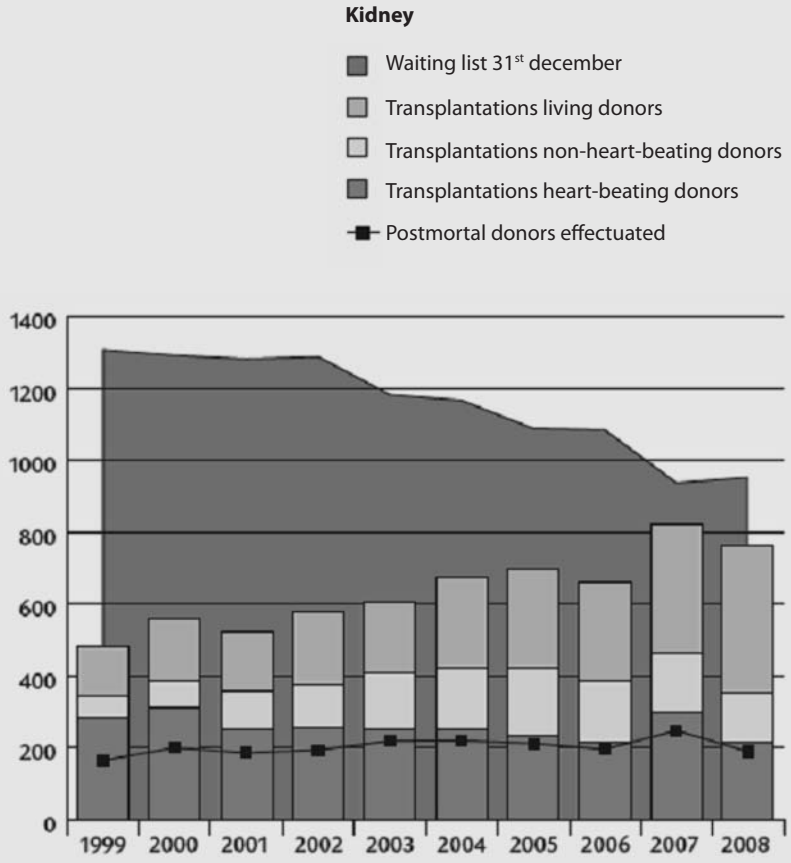
The first successful organ transplantation was a kidney transplantation between identical twins in Boston in 1954 [1-3]. Several years later, chemical immunosuppression with corticosteroids and azathioprine enabled transplantation between nonidentical individuals. Since 1966, kidney transplantations have also been performed in the Leiden University Medical Center (LUMC), the Netherlands. The introduction of new immunosuppressive agents and improvements in surgical techniques and post-transplant care made organ transplantation a routine and preferred therapy for treatment of end-stage renal, cardiac, hepatic and pulmonary failure [3] and pancreatic transplantation provides similar benefits for diabetic patients [4].

Currently, there are believed to be more than one million individuals worldwide with an organ allograft [5], and this number will further increase. However, the success is complicated by several problems, including the discrepancy between the demand for and the supply of organs and the need for continuous immunosuppressive medication. In the Netherlands, roughly 1200 patients are on the waiting list for organ transplantation and the mean time to kidney transplantation is approximately 4 years (figure 1). Complications from graft-preserving immunosuppression include an increased risk of malignancies [6], and of fungal, viral and parasitic infections [7, 8]. This chapter will provide a background of current knowledge of post-transplant cancer, with a focus on skin cancer. Furthermore, the increased incidence of other skin diseases in organ transplant recipients (OTR) will be discussed.

Incidence of cancer in organ transplant recipients

In the first 4000 patients undergoing kidney transplantation, over 40 primary malignant neoplasms were reported [6]. The increased risk of malignancies in OTR has been consistently supported by subsequent studies [9-13]. The overall risk for any cancer can be estimated to be 2- to 5-fold greater in OTR than in the general population [13-17]. This increased incidence has been shown to predominantly result from the occurrence of 4 distinct tumor types, namely non-melanocytic skin cancer (NMSC), lymphoproliferative disorders, anogenital dysplasias and Kaposi's sarcoma [9, 14, 16-19]. Recent data have indicated that thyroid cancers can be added to the group of more frequent cancers following organ transplantation [20]. Smaller, but significant, increases in hepatocellular and kidney cancers and some sarcomas have been observed [9, 14-17, 19]. For many common cancers including lung, colon, breast and prostate, the risk has been reported

Figure 1 Kidney transplantations in the Netherlands



The number of patients on the waiting list for a kidney transplantation on the 31st December per year is presented in blue. The number of patients receiving a living,
non-heart beating or heart-beating kidney transplantation per year in the Netherlands are presented by bars. Source of information: www.transplantatiestichting.nl
jaarverslag 2008

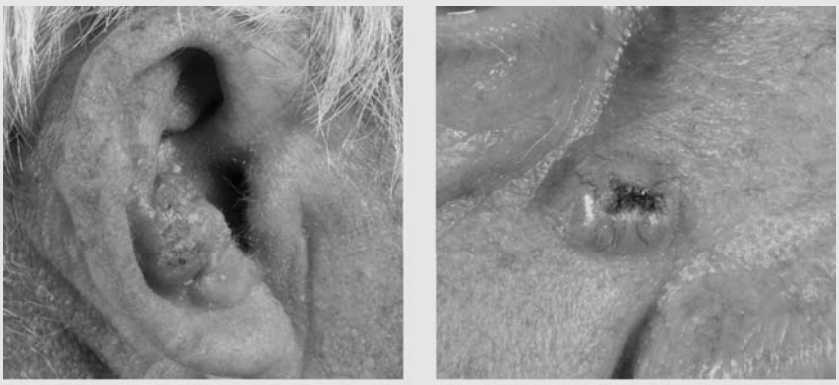
to be marginally or not significantly increased [12-14, 21]. Other studies have even shown a slightly reduced incidence of breast [22, 23] and prostate carcinoma [22].

Skin cancer

The incidence of malignant melanoma has been shown to be 3-fold elevated in OTR compared with the general population [22, 24]. Although low in absolute terms, the incidence of Kaposi's sarcoma represented a 200-fold higher risk [17]. The incidence of NMSC, including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), has reported to be roughly 55 times elevated [14, 25-28]. As this increased NMSC risk results in excessive number of patients with NMSC, we will focus on the development of NMSC in OTR.

NMSC is a collective term for SCC and BCC. SCC arise from malignant proliferation of the keratinocytes of the epidermis. The common clinical presentation of SCC is an erythematous keratotic papule or nodule that arises within a background of sun-damaged skin (Figure 2a). Lesions may ulcerate and have metastatic potential in around 5% [29]. BCC arise from the basal layer of epidermis. No universally accepted classification exists for BCC, but the most common variant, accounting for approximately 60% of all primary BCC presents as a raised, translucent papule or nodule with telangiectasias (Figure 2b). As the lesions enlarge ulceration may occur, but usually BCC do not metastasize [29].

Figure 2 Representative images of skin cancer **A**) Patient with a squamous cell carcinoma of the ear. **B**) Patient with a basal cell carcinoma on the cheek.



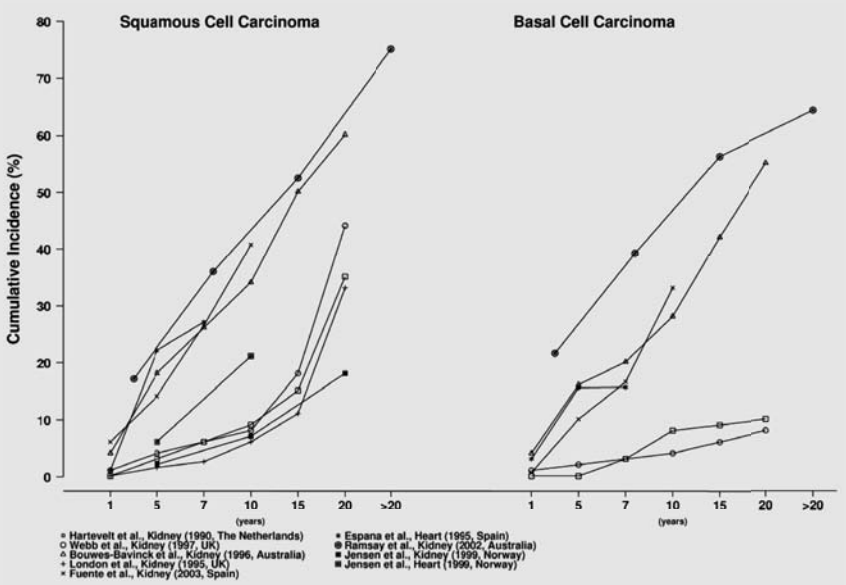
A B

The first report of increased NMSC in OTR came from Australia in the early 1970s, reporting seven patients with NMSC in a group of 51 kidney transplant recipients (KTR), which were immunosuppressed for up to 6 years [30]. Other studies from highly sun exposed areas in the USA and Australia followed [31-36], suggesting that sun exposure is an important risk factor for the development of NMSC. In OTR a predominance of SCC over BCC was shown [31-36], while in the general population BCC are more common than SCC. When reports of skin cancer in OTR in more temperate climates, such as Scandinavia, the Netherlands, Britain and Ireland, showed increased incidences of NMSC as well [9, 35, 37-43], it became more evident that limited sun exposure combined with immunosuppression can also result in the development of NMSC. A progressive increase in NMSC incidence with duration of immunosuppression was observed, indicating that immunosuppression is the key factor facilitating the development of NMSC in OTR [9, 39, 44-48]. Incidences of NMSC in OTR vary to a large extent from a 4- to 250-fold increased risk compared with the general population [39, 43]. Variability in the incidences between these studies may reflect that many factors play a role in NMSC development, including population differences in race, skin type, age, UV exposure and mean duration and type of immunosuppression. Furthermore, the variability in outcome may result from differences in the methods employed to determine the occurrence of NMSC. Some studies have reported incidence, others cumulative incidence, others relative risk, or the factor by which NMSC incidence is increased in OTR compared to a specified reference population. Yet others did not report the statistical methods used. We selected the population-based studies with high quality statistical analyses and summarized the data in Figure 3 and Table 1.

Several studies measured cumulative incidence of cutaneous SCC and BCC after organ transplantation (Figure 3). Bouwes Bavinck et al [44] and Ramsay et al [48] found equivalently high risks for SCC in the tropical Australian state of Queensland, with a cumulative incidence at 20 years of approximately 60% for both SCC and BCC (Figure 3). A study from Spain [46] only demonstrated cumulative incidence up to 10 years post-transplant, but showed a similar cumulative incidence compared with Australia (Figure 3). Meanwhile studies from the UK [49] and the Netherlands [39] found lower 20-year cumulative incidence rates for SCC of 34% and 40% respectively and 20-year cumulative incidence rates for BCC of 7% and 10%.

Another measure to express the incidence is the incidence rate per person years. The highest incidence rate that has been observed was 379 per 1,000 person years at risk for SCC and 127 per 1,000 person years for BCC in heart transplant recipients (HTR) in Australia [50] (Table 1). Studies from Spain, UK and The Netherlands found an incidence for SCC of 29/1,000, 71/1,000 and 7.6/1,000 person years respectively and for BCC of

Figure 3 Cumulative incidence of skin cancer in organ transplant recipients.



26/1,000, 22/1,000 and 3.3/1,000 person years [39, 45, 51]. To allow a proper comparison the incidence in the UK study (71/1,000) should be decreased by a factor 6, since this was the average number of cumulative SCC scored for given individuals [51].

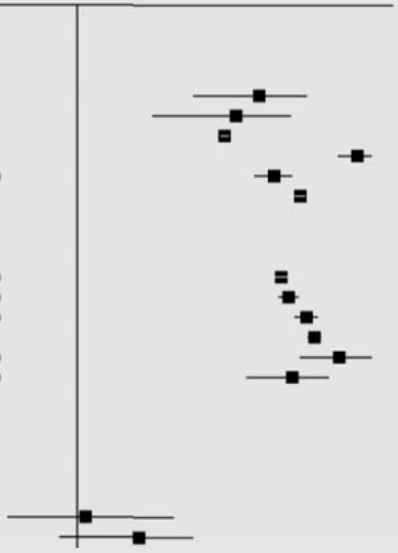
Other studies have provided incidence rates compared with the general population, presenting population-based standardized incidence ratios (SIR) [14-16, 27, 47]. To measure the SIR accurately, it is of importance that all cutaneous SCC and BCC are accurately reported to a comprehensive national cancer registry. The population-based SIR that were available for post-transplant SCC and post-transplant BCC are illustrated in Table 1. Based on these studies the risk for SCC is approximately 70 times increased and the risk for BCC 7 times increased compared with the general population.

Besides the incidence of NMSC it is of importance to determine the number of NMSC tumors per individual to measure disease burden and to design a more rational follow-up of these patients. Bouwes Bavinck et al [44] found an average of 10 NMSC tumors per OTR in Australia, Bordea et al [51] an average of 6 tumors per OTR in the UK, and Blohme et al [38] reported two OTR in Scandinavia with over 100 skin lesions each.

Table 1 Standardized incidence ratio.

HISTOLOGY/ORGAN STUDY (YEARS)	COUNT O/E	SIR (95%CI)	No. patients	Country or city / Comparison group	Standardised by sex/age/reference years
SQUAMOUS CELL CARCINOMA					
KIDNEY TRANSPLANTATIONS					
Hoxtell et al, 1977 (68-74)	First	4/0.11	36 (10-93)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72
Kinlen et al, 1979 (70-78)	First	3/0.13	23 (4-68)	3823	UK/Birmingham CR*	Y/5yrs/74
Gupta et al, 1986 (66-84)	NK	NK	18 (17-20)	523	Toronto / Detroit	Y/1yr/77-78
Hartevelt et al, 1990 (66-88)	All	38/0.15	253 (172-334)	764	Leiden / Eindhoven CR	Y/5yrs/83-85
Gjersvik et al, 2000 (83-32)	NK	NK	49 (33-70)	1020	Norway / CR	Y/1yr/calendar(1yr)
Moloney et al, 2006 (94-01)	All	282/3.4	82 (73-91)	1558	Ireland / CR	Y/1yr/94-200†
OTHER ORGANS +/- KIDNEY TRANSPLANTATIONS					
Adami et al, 2003 (70-97)	First	278/4.95	56 (50-63)	5931	Sweden / National CR	Y/1yr/calendar(1yr)
Jensen et al, 1999 (63-92)	First	97/1.49	65 (53-79)	2561	Oslo / National CR	Y/1yr/calendar(1yr)
Lindelof et al, 2000 (W) (70-94)	All	77/0.8	93 (73-116)	5356	Sweden / National CR	Y/1yr/calendar(1yr)
(M)		248/2.3	109 (95-123)			
Gjersvik et al, 2000 (83-32)	NK	NK	177 (81-336)	148	Norway / CR	Y/1yr/calendar(1yr)
Haagsma et al, 2001 (79-96)	First	7/0.1	70 (28-144)	174	The Netherlands / Mixture**	Y/1yr/calendar(1yr)
BASAL CELL CARCINOMA					
KIDNEY TRANSPLANTATIONS					
Kinlen et al, 1979 (70-78)	First	1/0.85	1.2 (0-7)	3823	UK / Birmingham	Y/5yrs/74
Hoxtell et al, 1977 (68-74)	First	3/0.88	3 (0.7-10)	495	Minnesota /Mineapolis-St Paul (survey)	Y/10yrs/71-72

SIR & 95% CI



The prevalence of OTR with multiple skin lesions was shown to vary between studies from 26 to 80%, which may be due to geographic differences, but also due to differences in length of follow-up and patient age [27, 33, 38, 39, 52-55]. According to a Scandinavian study, 25% of patients with a first NMSC have a second lesion within 13 months, and 50% have a second lesion within 3.5 years [27]. Liddington et al reported a mean interval of 15 months between detection of the first and second NMSC, and 11 months between the second and the third NMSC [42]. A French study showed that 34% of HTR and 52% of KTR with a first SCC developed a subsequent SCC within 3 years after the first SCC. After 5 years these percentages had risen to 64 and 67% in HTR and KTR, respectively [52]. A study from New Zealand showed that virtually all KTR with skin cancer developed multiple NMSC, with incidences of 30%, 50%, 60% and 80% at 1, 2, 3 and 5 years, respectively, after the first skin cancer [53]. These percentages are high compared with the general population, since the 3-year cumulative risk of a subsequent SCC after a first SCC in the general population has been described to be 18% [56]. While the risk for secondary SCC has been investigated in OTR, the risk of a subsequent BCC after the first BCC has not been reported in OTR. In the general population, approximately 50% of patients routinely treated for BCC developed multiple primary BCC during 10 years of observation [57, 58]. A meta-analysis of 7 independent studies showed a mean 3 year risk of 44% after an initial diagnosis of BCC [56].

Non-cutaneous malignancies

Large population-based cohort studies have reported that a range of non-cutaneous malignancies (NCM) occurs at increased rates in OTR, with an overall 2- to 5-fold increased cancer risk compared with the general population [13, 14, 16-18]. Among NCM we also count cancers of the mucous tissues. Anogenital dysplasias, comprising carcinoma of the vulva and anus, were 23- and 7-fold increased, respectively. The rate of lymphoproliferative disorders has been reported to be increased with a SIR of 7 for non-Hodgkin lymphoma [13, 14, 17, 18] and 4, 3 and 2 for Hodgkin's lymphoma [13, 14, 17, 18], multiple myeloma [13, 14, 17] and leukemia [13, 14, 17], respectively. Rates of liver and stomach cancer as well as epithelial lung cancer were approximately 2-fold increased. Most other common epithelial cancers, such as breast, prostate, ovarian and colorectal cancers, occurred at the same rate as in the general population [13, 14, 16-18]. Follow-up times of these studies were approximately 20 years.

Association between skin cancer and non-cutaneous malignancies in organ transplant recipients

In immunocompetent patients with cutaneous SCC, a 2-fold increased risk of NCM has been observed [59-61]. However, other studies did not show an overall increased risk of NCM in SCC patients [62]. In BCC patients, the overall cancer incidence has also been reported to be significantly elevated [61, 63, 64]. Vice versa, the occurrence of SCC as second primary malignancy after any NCM has been described to be increased in the general population [65]. Furthermore, Brennan et al showed an increased risk of NMSC after non-Hodgkin lymphoma [66]. The fact that cancer patients were at an increased risk for new primary cancers, may be explained by a common pathogenic pathway involved in the different types of cancer, and lifestyle factors of the patient, such as UV exposure, smoking and diet [67]. It is unknown whether the development of cutaneous SCC and/or BCC is associated with an increased risk of NCM in OTR as well, like in immunocompetent patients.

Risk factors for skin cancer in organ transplant recipients

The best-studied factors that appear to favor development of skin cancer are age at transplantation, male sex, fair skin type, high UV exposure, the presence of actinic keratoses, and the length and level of immunosuppression. Few investigators found all of these to be independent risk factors, but they were consistently reported across a wide range of studies [27, 28, 40, 44, 46, 47, 51, 68-71]. In a prospective study examining the first 3 years of immunosuppression in KTR from Spain, Ferrándiz [69] found a cumulative risk for NMSC of 18% with age at transplantation and occupational UV exposure being significant risk factors. Naldi from Italy [70] found age at transplantation and male sex to be the most important risk factors. Also from Italy, Caforio [68] found age at transplantation, fair skin type, high UV exposure, actinic keratoses and a high rejection score to be independently associated with an increased SCC risk in HTR. Since cumulative immunosuppressive load is difficult to calculate, a high rejection score in the first year post-transplantation was proposed to be a useful predictor for patients at risk. However, other studies did not confirm the association between number of rejections and development of NMSC in OTR [51, 70, 72, 73].

The presence of human papillomavirus (HPV) has been suggested to be a risk factor for SCC, although a causative role for HPV in skin cancers in OTR has not been proven. HPV DNA was found in 65% to 90% of skin cancers that developed in OTR [74-76], while in immunocompetent individuals approximately in 40% of the skin cancers HPV

DNA was found [75, 77, 78]. The rate of HPV detection in normal sun-exposed skin has been described to be higher in OTR with skin cancer compared with those without skin cancer. This supports the hypothesis that OTR have persistent HPV infection that predisposes to oncogenesis [79]. However, HPV is also frequently present in the hair follicles and normal skin from OTR [80]. Furthermore, comparing OTR with and without skin cancer, others have shown an equally high prevalence of HPV DNA in keratotic skin lesions in both groups of patients, and a similar detection rate and spectrum of HPV infection in hyperkeratotic papillomas and actinic keratoses [81]. Recent epidemiological [77, 82] as well as experimental studies [83] have suggested a possible synergetic effect between HPV infection and UV radiation in carcinogenesis of the skin. Two major risk factors for skin cancer in OTR, UV exposure and prolonged immunosuppressive therapy, will be discussed in more detail below.

Ultraviolet radiation

UV exposure is the primary risk factor for NMSC both in the general population [84] and in OTR [68, 85]. This is illustrated by an increased risk of skin cancer in patients with high sun exposure before organ transplantation [46, 68, 86]. Furthermore, the cumulative risk for SCCs was reported to be greater in countries with a high level of UV radiation, such as Australia (34% at 10 years) [44] or Spain (33% at 10 years) [46], compared with countries with limited sun exposure, such as the Netherlands and Norway (7% at 10 years) [39, 47]. The preferential location of SCC on sun-exposed areas further supports the pathogenic role of sunlight [39]. It is assumed that the oncogenic properties of UV radiation are due to a direct mutagenic effect and an immunosuppressive effect. It has been shown that UV light is a keratinocyte mutagen, which can cause mutations, such as cytosine to thymine transitions at cytosine-containing dipyrimidine sites [87]. When these mutations affect the function of sufficient oncogenes, tumor-suppressive genes, and important housekeeping genes, outgrowh of neoplastic keratinocytes can occur. UV-induced immunosuppression is a highly complex process and several different pathways are involved [84, 88-90]. In particular, low doses of UV light radiation reduce the number and function of epidermal Langerhans' cells, impairing their role in the immune response against virus-infected cells and transformed cells. UV light radiation can also induce systemic immunosuppression by inducing the generation of soluble mediators, notably cis-urogenic acid and interleukin-10 [84, 88-90].

Immunosuppressive therapy

The maintenance immunosuppressive therapy in OTR usually consists of prednisone in combination with immunosuppressants such as azathioprine (purine-antagonist),

mycophenolate mofetil (inosinemonophosphatehydrogenase-inhibitor), cyclosporine or tacrolimus (calcineurine-inhibitors), and sirolimus or everolimus (mTOR-inhibitors). Acute rejection in OTR will usually be treated with high doses of polyclonal antibodies against thymocytes (ATG) and monoclonal antibodies against CD3 (muromonab). In hairless mousemodels it has been shown that classical immunosuppressants, azathioprine and cyclosporine, speeds up UV carcinogenesis and adversely affects repair of UV-induced DNA-damage in skin cells [91]. Moreover, Azathioprine has been reported to induce selective UVA photosensitivity, thus increasing the DNA damage caused by UV exposure [92]. Cyclosporine can impair UV-induced apoptosis, which also increases the risk of skin cancer [93]. In contrast to the traditional immunosuppressants, mycophenolate mofetil and sirolimus, did not enhance UV carcinogenesis [94]. Although mycophenolate mofetil, like azathioprine, interferes with purine synthesis, it does not give rise to incorporation of (6-thio-guanine) pseudobases that photosensitize DNA. Furthermore, sirolimus operates through an entirely different mechanism by blocking mTOR (mammalian target of rapamycin), which has been shown to have an antiangiogenic effect, resulting in impaired tumor outgrowth [94, 95]. However, so far, there is no convincing clinical evidence for differences in oncogenic potential between the specific immunosuppressive agents. Comparison of incidence rate by type of immunosuppressive drug is difficult, because the regimen of immunosuppressive agents is strongly associated with the time period in which the patient is transplanted. A recent study showed that treatment with azathioprine was associated with a significant increased risk for SCC [96]. Evidence also suggests that sirolimus, a mTOR inhibitor, compared with other immunosuppressive medications may confer a decreased risk of skin cancer [97, 98].

Rather then the type of immunosuppressive agent, the total level of immunosuppression may determine the risk of skin cancer [44, 70, 99, 100]. In a prospective trial in which patients were randomly assigned, KTR receiving low dose cyclosporine regimen had a significantly lower incidence of secondary skin cancers compared with the patients using normal dose cyclosporine [68]. Furthermore, the greater degree of immunosuppression after heart transplantation, to prevent the catastrophic rejection of the donor organ, has been shown to result in a higher incidence of skin cancer in HTR compared with KTR [47, 54, 101, 102].

Other skin diseases in organ transplant recipients

Besides skin cancers, also benign skin tumors [28, 39, 44, 47] and fungal, viral, and bacterial skin infections [103-105] are frequently observed in OTRs. The prevalence of skin infections is very high and several studies have described that 55% to 97% of OTR do have some type of infection [104-108]. The spectrum of skin infections differs according to the post-transplant time period [105]. During the first month post-transplant, infections mainly result from surgical interventions [103]. After the first month post-transplant, infectious skin diseases are more frequently a result of severe immunosuppression, manifesting in infections with herpes viruses (herpes simplex virus, varicella-zoster virus, cyto-megalovirus, Epstein-Barr virus), yeasts (Candida), and bacteria [105]. Six months and more after transplantation, the chronic and progressive infections start to exert clinically significant effects [103, 105], of which infections with HPV have been most frequently described [79, 103, 109]. Compared with the large number of studies focusing on the development of malignant and benign skin tumors in OTR, infectious and inflammatory skin diseases were only studied scarcely [79, 103-109].

Aim and structure of the thesis

The aim of the studies presented in this thesis is broadly twofold. Firstly, we aimed to determine the pattern and frequency of SCC, BCC, NCM and skin diseases in OTR transplanted in the Leiden University Medical Center *(descriptive epidemiology)*. Increasing the recognition of these clinical complications can help to provide a rationale for more extensive follow-up of OTR and allow more rapid clinical interventions. Secondly, we aimed to identify causes for the increased incidence of malignancies in OTR *(analytic epidemiology)*. Identification of the risk factors involved in the development of SCC, BCC, and NCM may increase the efficiency of OTR follow-up.

Chapter 2 describes the standardized morbidity ratio of NCM, SCC and BCC in KTR who had received a transplantation at the Leiden University Medical Center between 1966 and 2006.
Chapter 3 determines the risk to develop a second SCC or BCC following the occurrence of the first SCC or BCC in a cohort of KTR and studies risk factors for the development of subsequent SCC or BCC.
Chapter 4 investigates the frequency and number of registered skin diseases in OTR

transplanted between 1966 and 2006 in a single centre, which were diagnosed between 1994 and 2006. Furthermore, the relative contributions of the different skin diseases in relation to the number of years after transplantation were studied.

Chapter 5 compares the cumulative incidence of skin cancer in SPKTR with the cumulative incidence of skin cancer in KTR in relation to potential risk factors of skin cancer.

Chapter 6 studies the risk of NCM after the development of cutaneous SCC and/or BCC in KTR.

Chapter 7 studies whether the number of transplantations, as a marker for the rejection status of the patient, is associated with the risk of the development of malignancies. The risk for cutaneous SCC and other malignancies are analyzed separately.

Chapter 8 summarizes and discusses the results described in the preceding chapters.

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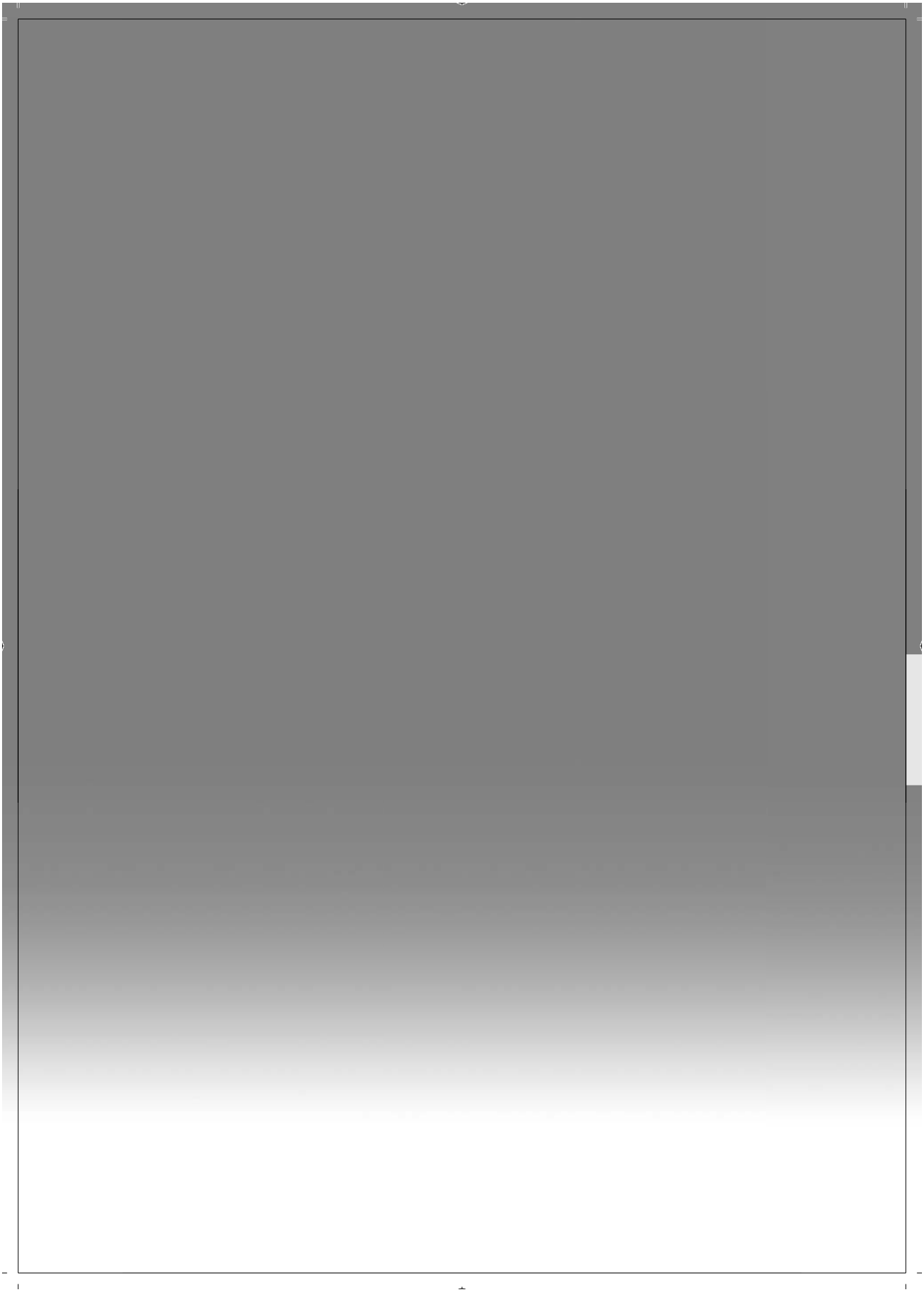

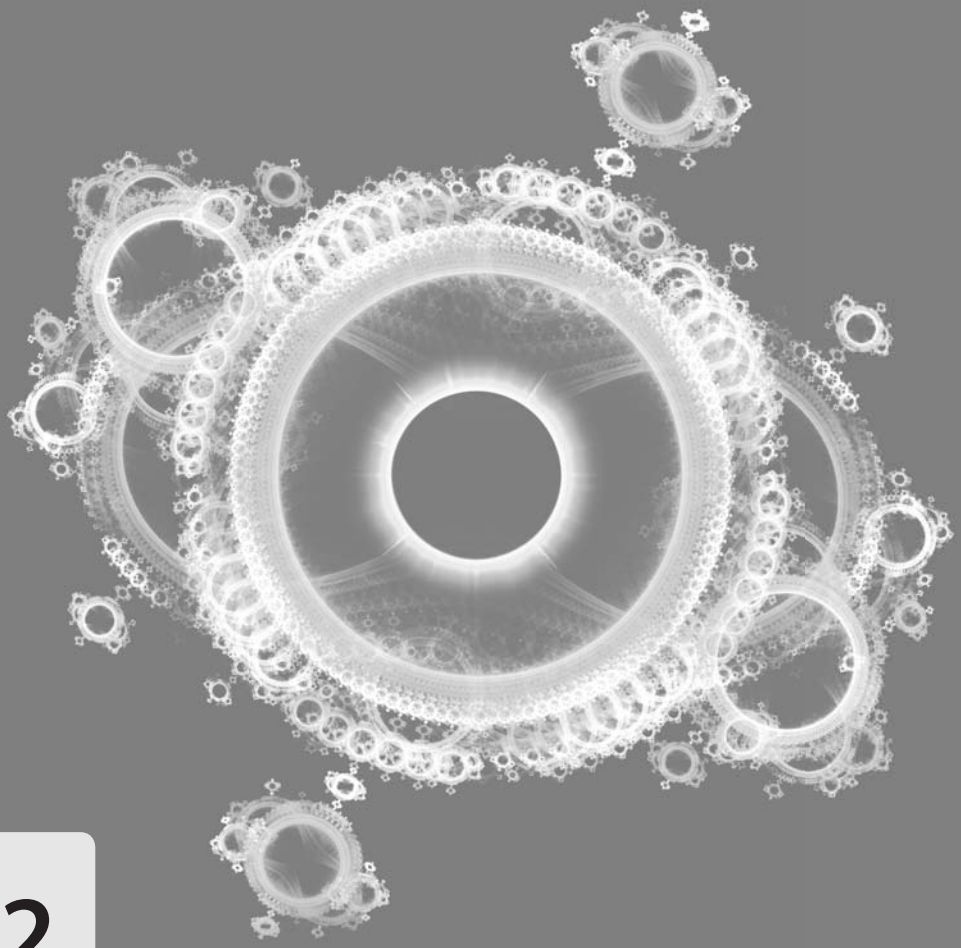

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Incidence of cancer in kidney transplant recipients: a long-term cohort study in a single center

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Cancer Epidemiology 2010, in press

Abstract

In a long-term cohort study, we calculated cancer incidences and survival rates after the development of these cancers in kidney-transplant recipients. The cancer incidences were compared with those in the general population. The occurrence of cancer was recorded in all patients who received a kidney transplantation between 1966 and 2006. The median follow-up time was more than 9 years with a maximum of almost 40 years. Altogether 327 (17%) of 1906 patients developed cancer after transplantation: 142 (7%) had non-cutaneous malignancies; 178 (9%) cutaneous squamous-cell carcinomas and 138 (7%) basal-cell carcinomas. The cumulative incidence of any cancer was 13%, 33% and 47% after 10, 20 and 30 years, respectively. The incidences of cancers of the oral cavity, stomach, female genital organs, kidney, thyroid gland, leukemias and lymphomas, and cutaneous squamous-cell carcinoma were significantly increased with a highest standardized morbidity ratio of 40 for cutaneous squamous-cell carcinomas. Survival rates after non-cutaneous malignancies were 57%, 43% and 36% and after non-melanocytic skin cancer 99%, 90% and 77% after 1, 3 and 5 years, respectively. The increased incidence of non-cutaneous malignancies after kidney transplantation is associated with a high mortality. Prevention of cancer after kidney transplantation should be a major focus of future research.

Introduction

There is abundant evidence that the incidence of cancer is increased in kidney-transplant recipients (KTR) [1-4]. The risk for the most common malignancies, e.g. colon, lung, stomach, oesophagus, pancreas and ovary cancers is, generally, threefold increased in KTR compared with the general population [1, 3, 5]. Cancers associated with viral infections, such as cervical cancer, lymphoma [6, 7], Kaposi sarcoma [8, 9] and skin cancers, in particular cutaneous squamous-cell carcinomas (SCC) [10-13] appear to be increased the most. Recently, thyroid cancers were added to the group of high cancer risk following organ transplantation [14]. However, not all cancers are increased in the transplant population [5, 15]. Breast [5, 16] and prostate [5] malignancies are two of the most common cancers in the general population that are not increased in KTR. Despite considerable evidence that the incidence of cancer is increased in KTR, a recent study of Kiberd et al. showed that the overall mortality rates are not substantially different compared with the mortality in the general population [17].

The aim of this study was to estimate the incidence of non-cutaneous malignancies (NCM) and cutaneous SCC and basal-cell carcinoma (BCC) in all patients who had received a kidney transplantation at the Leiden University Medical Center (LUMC) and to compare this incidence with the incidence in the general Dutch population. We also assessed the survival rate of KTR who had NCM before transplantation and the survival rates after the development of post-transplant NCM, cutaneous SCC and BCC.

Patients and methods

Patients

We performed a retrospective cohort study of all 1906 patients who received a first kidney transplantation at the LUMC between March 1966 and January 2006. Most of these patients were regularly followed at the department of Nephrology. When patients had cutaneous problems they were also seen at the department of Dermatology. At each visit to the skin clinic the entire skin was checked for skin problems. Special attention was focused on the possible presence of keratotic skin lesions and skin cancers. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Between 1966 and 1986, the immunosuppressive treatment of KTR in our clinic consisted of duo therapy with prednisolone (P) and azathioprine (Aza), but shortly

after 1986 all new KTR were immunosuppressed with P and cyclosporine A (CsA). After 1996 triple therapy became the treatment of choice where, initially, most new KTR were treated with P, mycofenolatemofetil (MMF) and CsA and later with P, MMF and tacrolimus (Tac).

KTR, in whom acute graft rejections were observed, were usually initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection a second rejection treatment with anti-thymocyte globulin (ATG) and a third rejection treatment with once more methylprednisolone were given. In exceptional cases muronomab-CD3 (OKT3) was given when a fourth rejection treatment was needed. With the exception of some rare patients, induction treatments with ATG and/or OKT3 were not given to KTR who were transplanted in the LUMC. Starting in 2000, however, induction treatment with basiliximab became common practice.

Collection of data

Data recorded for all KTR included the date of the first and subsequent transplantations, dates of birth, sex, and the dates of cancer, death or last follow-up. The main outcomes of cancer were the diagnoses of NCM and cutaneous SCC and/or BCC and were collected from the computerized oncological registry of the LUMC, the database from the department of Pathology and the national histological database (PALGA). PALGA is an acronym, literally translated: pathological anatomy national automated archive. Excerpts of all histopathology and cytopathology reports are generated automatically at the participating laboratories and transferred to the central databank. Both the decentralized systems and the central system perform checks on the quality and completeness of excerpts. This central databank contains about 42 million records on almost 10 million patients [18]. The medical charts were also hand searched for the diagnosis of cancer. Premalignant and in situ lesions were excluded. Follow-up data were collected until June 2007, the arbitrary end of the study.

The diagnoses of NCM were based on the International Classification of Diseases 10th Modification Diagnoses Codes (ICD-10). NCM were categorized into carcinomas, lymphomas, leukemias, sarcomas and an "undefined" group. Locations of the NCM were categorized as: head and neck; digestive organs; lower respiratory system; bone and soft tissues; skin; breast; female genital organs; male genital organs; urinary tract; central nervous system; endocrine glands; blood, bone marrow and lymph nodes; eye and orbit, other sites; and unknown primary site. Different than in the ICD-10 classification we classified lip carcinomas as cutaneous SCC or BCC and not as NCM.

Statistical data for cancer per 5 year age categories were obtained from the Eindhoven Cancer Registry for the period 1966-1988 and from the Netherlands Cancer

Registry for the period 1989-2006. There are eight comprehensive cancer centers in the Netherlands who collect data of new cancer patients, such as tumor type, incidence date and stage. The Netherlands Cancer Registry was established in 1989 and provides incidence data on a national level. This registry contains data on nearly all new cancer cases in the Netherlands. The data are collected by co-workers of the regional comprehensive cancer centers.

Statistical analyses

Kaplan Meier survival analyses were used to estimate the cumulative incidences of cancer after transplantation. As opening dates for these analyses we used the date of the first transplantation; as closing dates we used the date of diagnosis of the first specific malignancy, the date of the patient's death or the date of last follow up. Malignancies before transplantation were not considered in these analyses.

The incidence of cancer in the KTR after transplantation was compared with the incidence in the general population by calculating standardized morbidity ratios (SMR) with 95% confidence interval and was matched for age, sex and time period in which the malignancy had occurred. The SMR for haematolymphopoetic malignancies was calculated for the total group since these malignancies were not registered for the different subcategories during the earlier periods. The expected number of BCC could not be calculated, since this type of cancer is not routinely registered in the Netherlands. If a patient had developed two NCM after transplantation, person years between the transplantation and the first NCM and between the first and second NCM were calculated. In patients with multiple cutaneous SCC and BCC only the first occurrence after transplantation was considered.

Kaplan Meier survival analyses were used to estimate survival of the patients after cancer. As opening dates for these analyses we used the date of the specific malignancy; as closing dates we used the date of the patient's death or the date of last follow up. A Cox proportional hazard analysis was used to calculate the chance of decreased survival after transplantation of the patients with a pre-transplant malignancy compared with the other patients. Survival of the patients was not compared with survival in the general population, because in the KTR the stage of the disease, which is essential for the comparison of survival, was not systematically collected.

The statistical calculations were performed using SPSS for Windows version 16.0.1 (SPSS Inc, Chicago, IL) and Stata/SE for Windows version 10.1 (Stata Corp LP, College Station, Texas).

Results

Baseline characteristics of the KTR

The median age at transplantation of the 1906 KTR was 43.9 years (range 3.8 – 77.5) with a median follow up of 9.2 (range 0 -39.9) years. A total of 1175 (61.6%) out of 1906 KTR were male. Altogether 50 (3%) of the patients already had a history of cancer before transplantation and 327 (17%) developed cancer after transplantation: 142 (7%) had NCM; 178 (9%) cutaneous SCC and 138 (7%) BCC.

The cumulative incidence of any malignancy after transplantation was 13% after 10 years, 33% after 20 years and 47% after 30 years (Figure 1). Table 1 shows characteristics of the 50 KTR with cancer before the transplantation and Table 2 of the 327 KTR who developed their first cancer after the kidney transplantation.

Description of malignancies

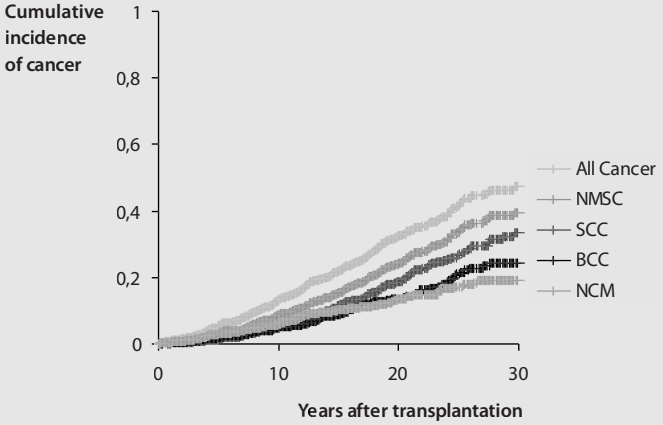
Forty-one of the 53 malignancies (in 50 patients) before transplantation were NCM, of which 11 were a malignancy of the kidney (Table 1). Five out of 41 patients with a first NCM before transplantation also had a second NCM before transplantation (Patients 1 to 5 in Table 3). In 3 of these 5 patients there was a malignancy of both kidneys.

Of the 46 NCM before transplantation 42 were carcinomas, 1 was a non-Hodgkin lymphoma, whereas the cellular type was undefined in 3 NCM (Tables 1 and 3). The most frequent locations of the first and second NCM before transplantation were the urinary tract (17 times), followed by the digestive organs (7 times), breast (7 times) and the female (6 times) and male (4 times) genital organs (Tables 1 and 3).

After the kidney transplantation a total of 142 KTR developed a NCM (Table 2), of which 6 had already a NCM before transplantation (Patients 6 to 11 in Table 3). Of the 136 patients with a first NCM after transplantation 9 developed a second NCM (Patients 12 to 20 in Table 3).

Of the 151 NCM after transplantation 112 were carcinomas, 8 leukemias, 22 lymphomas and 2 sarcomas whereas the cellular type was undefined in 7 NCM (Tables 2 and 3). The most frequent locations of the first and second NCM after transplantation were the digestive organs (45 times), followed by the respiratory tract (15 times), the urinary tract (14 times), the female genital organs (12 times), bone marrow (12 times) and the male genital organs (11 times). The leukemias consisted of 4 acute myeloid leukemias, 1 chronic myeloid leukemia and 3 chronic lymphocytic leukemias (2 B-cell, 1 T-cell). The lymphomas consisted of 20 non-Hodgkin lymphomas (19 B-cell, 1 T-cell), 1 classical Hodgkin lymphoma and 1 non-characterized lymphoma.

Figure 1 Cumulative incidence of any malignancy, non-melanocytic skin cancer (NMSC), squamous-cell carcinoma (SCC), basal-cell carcinoma (BCC) and non-cutaneous malignancy (NCM) in kidney transplant recipients. The numbers of patients at risk are indicated in the Table.



Numbers of KTR at risk at the specified time periods after transplantation (yrs)

Type of skin cancer	0	10	20	30
All cancer	1857	793	271	55
NMSC	1898	818	283	56
SCC	1904	844	301	63
BCC	1898	850	323	69
NCM	1865	863	354	85

Cutaneous SCC and BCC were, by far, the most frequently diagnosed cancers after transplantation (Table 2). SCC was diagnosed in 178 and BCC in 138 patients, respectively. The maximum number of SCC in one patient was 68 and the maximum number of BCC in one patient was 28. In total there were more than 1800 SCC and BCC in these patients. For this study, however, only the first SCC and BCC were considered.

In total, 29 SCC and 8 BCC of the lip had been diagnosed in 31 KTR. In 8 of these patients SCC of the lip was the first presentation of SCC and in 7 patients BCC of the lip was the first presentation of BCC.

Table 1 Characteristics of transplant recipients who developed the first malignancy before the kidney transplantation.

Type of cancer (cell type)$	No.*	Male N (%)	Age at TP* (yrs) Median (25%;75%)#	Age at Cancer (yrs) Median (25%;75%)	Time before TP* (yrs) Median (Max;Min)
Digestive organs	6	4 (67)	59.9 (48.2;65.3)	53.6 (38.5;59.5)	-3.5 (-27.5;-0.2)
Colon	*4*	*3 (75)*	*55.8*	*53.6*	*-2.3*
Recto sigmoid	*2*	*1 (50)*	*61.8*	*44.9*	*-16.9*
Breast	7	0 (0)	50.8 (44.5;70.6)	43.7 (37.7;64.3)	-6.7 (-14.0;-4.7)
Female genital organs	6	0 (0)	54.6 (50.5;57.6)	44.4 (41.2;48.1)	-9.4 (-21.5;-6.4)
Cervix uteri	*3*	*0 (0)*	*54.7*	*44.6*	*-7.3*
Corpus uteri	*3*	*0 (0)*	*54.4*	*44.2*	*-11.4*
Male genital organs	3	3 (100)	63.4	60.0	-2.9
Prostate	*3*	*3 (100)*	*63.4*	*60.0*	*-2.9*
Urinary tract	13	11 (85)	59.4 (52.6;66.4)	47.7 (40.2;60.9)	-6.5 (-21.6;-1.2)
Kidney	*11*	*9 (82)*	*59.4 (53.4;67.7)*	*51.8 (41.9;61.1)*	*-6.5 (-17.7;-1.2)*
Urinary bladder	*2*	*2 (100)*	*56.0*	*42.3*	*-13.7*
Central nervous system	3	2 (67)	38.0	24.9	-10.4
Brain (undefined)	*3*	*2 (67)*	*38.0*	*24.9*	*-10.4*
Endocrine glands	2	2 (100)	55.6	52.7	-2.8
Thyroid gland	*2*	*2 (100)*	*55.6*	*52.7*	*-2.8*
Haematolymphopoetic	1	0 (0)	41.8	33.0	-8.8
Stomach (lymphoma)	*1*	*0 (0)*	*41.8*	*33.0*	*-8.8*
All non-cutaneous malignancies together	41	22 (54)	54.9 (46.3;64.1)	46.0 (37.8;59.8)	-6.5 (-34.3;-0.2)

Non-melanocytic skin cancer	8	4 (50)	60.6	(54.1;65.2)	57.8	(50.6;63.0)	-1.3	(-8.9;-0.2)
Squamous-cell carcinoma	*2*	*1 (50)*	*65.1*		*64.3*		*-0.8*	
Basal-cell carcinoma	*8*	*4 (50)*	*60.6*	*(54.1;65.2)*	*57.8*	*(50.6;63,2)*	*-1.3*	*(-8.9;-0.2)*
Other skin cancer	2	0 (0)	54.6		53.1		-1.4	
Malignant melanoma	*2*	*0 (0)*						
			54.6		*53.1*		*-1.4*	
All patients with malignancies together	50	26 (52)	55.5	(46.4;64.9)	47.8	(38.4;60.1)	-6.2	(-9.9;-2.8)

$All malignancies were carcinomas unless otherwise indicated.
*Some patients had more than one type of malignancy.
**TP = kidney transplantation.
25%;75% were only shown when the number of malignancies were 5 or more

Table 2 Characteristics of transplant recipients who developed the first malignancy after the kidney transplantation.

Type of cancer (cell type)$	No.*	Male N (%)	Age at TP** (yrs) Median (25%;75%)#		Cumulative incidence (%) after different periods after transplantation(yrs)			
					5	10	20	30
Head and neck	9	6 (67)	51.0	(38.7;56.2)	0.1	0.4	0.9	1.6
Oral cavity	*5*	*2 (40)*	*51.0*	*(35.2;57.1)*	*0.1*	*0.2*	*0.7*	*0.7*
Salivary glands	*2*	*2 (100)*	*48.6*					
Nasal cavity (undefined)	*1*	*1 (100)*	*40.5*					
Larynx	*1*	*1 (100)*	*51.2*					
Digestive organs	27	14 (52)	47.5	(37.5;51.2)	0.5	0.8	2.9	5.9
Colon	*10*	*2 (20)*	*42.7*	*(36.5;50.6)*	*0.1*	*0.1*	*0.9*	*3.4*
Stomach	*8*	*7 (88)*	*50.8*	*(38.0;55.4)*	*0.2*	*0.2*	*1.2*	*1.6*
Gallbladder or pancreas	*4*	*3 (75)*	*53.0*					
Esophagus	*3*	*1 (33)*	*35.8*					
Small intestin	*1*	*1 (100)*	*47.9*					
Anus	*1*	*0 (0)*	*47.5*					
Lower respiratory system	17	12 (71)	50.1	(46.0;56.8)	0.5	1.1	1.9	2.5
Lungs	*16*	*11 (69)*	*50.6*	*(46.0;57.0)*	*0.5*	*1.1*	*1.7*	*2.4*
Heart (sarcoma)	*1*	*1 (100)*	*47.4*					
Bone and soft tissue (sarcoma)	1	1 (100)	31.1					
Breast &	7	0 (0)	42.6	(36.3;43.8)	0.4	0.6	1.9	1.9
Female genital organs &	10	0 (0)	44.7	(29.2;54.0)	0.5	1.1	3.1	5.1
Vulva	3	*0 (0)*	*23.0*					
Cervix uteri	3	*0 (0)*	*32.1*					
Corpus uteri	3	*0 (0)*	*44.8*					
Ovary	1	*0 (0)*	*65.3*					

Male genital organs &	11	11 (100)	56.3	(49.0;71.4)	0.6	0.9	1.3	1.8
Prostate (1x undefined)	*8*	*8 (100)*	*64.8*	*(54.1;71.7)*	*0.4*	*0.7*	*1.1*	*1.1*
Testis (2x undefined)	*3*	*3 (100)*	*33.3*					
Urinary tract	13	9 (69)	46.7	(30.6;52.9)	0.5	0.6	1.3	1.3
Kidney	*8*	*6 (75)*	*40.8*	*(25.8;52.3)*	*0.3*	*0.4*	*0.9*	*0.9*
Urinary bladder	*5*	*3 (60)*	*51.5*	*(44.2;58.1)*	*0.2*	*0.2*	*0.4*	*0.4*
Central nervous system	2	2 (100)	36.3					
Brain (undefined)	*2*	*2 (100)*	*36.3*					
Endocrine glands	5	3 (60)	34.6	(31.0;51.6)	0.2	0.4	0.4	0.8
Thyroid gland	*4*	*2 (50)*	*36.2*		*0.1*	*0.3*	*0.3*	*0.7*
Pituitary gland (undefined)	*1*	*1 (100)*	*34.6*					
Haematolymphopoetic	30	17 (57)	42.6	(28.6;51.6)	0.4	1.1	3.4	4.0
Leukemia	*8*	*4 (50)*	*27.5*	*(23.3;50.7)*	*0.1*	*0.2*	*0.9*	*1.3*
Lymphoma	*22*	*13 (59)*	*43.3*	*(32.3;53.3)*	*0.4*	*0.9*	*2.4*	*2.7*
Oral cavity	*1*	*1 (100)*	*11.3*					
Nasal cavity	*3*	*2 (67)*	*44.5*					
Stomach	*1*	*1 (100)*	*49.2*					
Bowel	*4*	*3 (75)*	*38.5*					
Liver	*1*	*1 (100)*	*28.7*					
Bone	*1*	*0 (0)*	*42.6*					
Bone marrow	*4*	*2 (50)*	*56.8*					
Abdomen and peritoneum	*2*	*2 (100)*	*43.3*					
Breast	*1*	*0 (0)*	*53.7*					
Lymph nodes	*4*	*1 (25)*	*39.7*					
Unknown primary site	4	3 (75)	39.7					
Non-cutaneous malignancies together	136	78 (57)	46.0	(34.9;53.2)	3.0	5.9	13.5	19.0

Table 2 Baseline characteristics of transplant recipients who developed the first malignancy after the kidney transplantation, continued.

Type of cancer (cell type)$	No.*	Male N (%)	Age at TP** (yrs) Median (25%;75%)#		Cumulative incidence (%) after different periods after transplantation(yrs)			
					5	10	20	30
Non-melanocytic skin cancer	231	152 (66)	40.4	(29.5;48.5)	3.0	8.7	24.4	39.5
Squamous-cell carcinoma	*178*	*120 (67)*	*39.2*	*(28.9;47.4)*	*1.4*	*5.8*	*18.9*	*33.4*
Basal-cell carcinoma	*138*	*92 (67)*	*41.3*	*(31.4;48.1)*	*1.9*	*4.9*	*13.7*	*24.3*
Other skin cancers	*22*	16 (73)	*43.2*	*(31.7;54.7)*	*0.6*	*0.7*	*2.1*	*3.7*
Malignant melanoma	*10*	*8 (80)*	*42.0*	*(26.9;53.7)*	*0.2*	*0.3*	*1.3*	*1.3*
Kaposi sarcoma	*4*	*3 (75)*	*45.7*					
Sweat gland carcinoma	*3*	*2 (67)*	*56.6*					
Dermatofibrosarcoma	*2*	*1 (50)*	*33.5*					
Sebaceous gland carcinoma	*1*	*1 (100)*	*32.9*					
Merkel cell carcinoma	*1*	*1 (100)*	*37.9*					
Hemangiopericytoma	*1*	*0 (0)*	*54.8*					
All patients with malignancies together	327	207 (63)	42.9	(31.3;50.9)	5.3	13.1	32.5	47.3
No malignancy at any time	1529	942 (62)	43.8	(32.3;54.8)				

$All malignancies were carcinomas unless otherwise indicated.
*Many patients had more than one type of malignancy.
**TP = kidney transplantation.
25%;75% were only shown when the number of malignancies were 5 or more.
& Cancers of the breast and the female genital organs were analyzed in women only and cancers of the male genital organs were analyzed in men only.

Table 3 Characteristics of patients with two non-cutaneous malignancies before and/or after the kidney transplantation.

Patient	Sex*	Age at TP * (years)	First cancer	Time related to TP (years)	Second cancer	Time related to TP (years)
1	M	60.2	Bladder carcinoma	-21.6	Kidney carcinoma	-2.5
2	M	53.8	Kidney carcinoma	-11.9	Kidney carcinoma	-5.9
3	M	41.2	Kidney carcinoma	-6.0	Kidney carcinoma	-6.0
4	M	62.2	Kidney carcinoma	-1.2	Kidney carcinoma	-1.2
5	M	67.7	Kidney carcinoma	-6.9	Prostate carcinoma	-1.9
6	F	72.9	Kidney carcinoma	-3.1	Kidney carcinoma	2.8
7	M	41.1	Bowel carcinoma	-0.2	Bowel carcinoma	23.4
8	F	50.5	Bowel carcinoma	-1.5	Lung carcinoma	4.8
9	F	44.5	Breast carcinoma	-6.6	Thyroid gland carcinoma	8.9
10	F	70.6	Breast carcinoma	-6.4	Stomach carcinoma	2.5
11	F	46.4	Endometrial carcinoma	-11.4	Lung carcinoma	0.7
12	F	49.1	Bowel carcinoma	7.3	Bowel carcinoma	18.2
13	F	40.3	Bowel carcinoma	3.2	Stomach carcinoma	22.0
14	F	38.5	Bowel carcinoma	23.3	Vulva carcinoma	29.1
15	F	43.8	Breast carcinoma	25.4	Breast carcinoma	34.9
16	F	61	Cervix carcinoma	8.7	Vagina carcinoma	9.2
17	M	63.1	Gallbladder/pancreas carcinoma	7.3	Unknown primary site carcinoma	7.3
18	M	52.1	Squamous cell carcinoma tongue	6.4	Lung carcinoma	13.7
19	M	53.2	Bone marrow lymphoma	2.0	Lung carcinoma	2.1
20	M	58.8	Bone marrow lymphoma	2.2	Pancreas carcinoma	3.2

*TP = kidney transplantation; M = male; F = female

Incidence of cancer in KTR compared with the general population

The SMRs adjusted for age, sex and the period of occurrence of the cancers after kidney transplantation are displayed in Table 4. Compared with the general population the risk to develop NCM was 1.6 times increased in KTR. The highest risks were found for thyroid carcinoma and haematolymphopoetic malignancies with SMRs of 9.5 and 4.1, respectively (Table 4). NCM of the oral cavity, stomach, female genital organs and kidney were also significantly increased compared with the general population (Table 4). The risks of prostate carcinoma and breast cancer in KTR were slightly decreased, but statistical significance was not reached (Table 4).

The risk of cutaneous SCC was 40 times, the risk of Kaposi sarcoma 21 times and the risk of malignant melanoma 3 times increased compared with the general population (Table 4). Because of incomplete registration of BCC in the general population, the SMR of BCC could not be calculated.

Survival of the patients after malignancies

Altogether 107 (75%) out of the 142 patients who had developed 1 or 2 NCM after transplantation and 116 (50%) out of the 231 patients who had developed skin cancer had died (Table 5). The survival rates after the diagnosis of NCM were 57%, 43%, 36% and 22% after 1, 3, 5, and 10 years, respectively (Table 5). Especially the diagnoses of stomach cancer and lung cancer were associated with a poor prognosis (Table 5). The patients with skin cancer survived much longer after the diagnosis of the first skin cancer with survival rates of 99%, 90%, 77% and 57% after the same time periods, respectively (Table 5).

The survival rate after transplantation of the 41 patients who had a history of a malignancy before transplantation was similar compared to the KTR without NCM before transplantation. The hazard ratio with 95% confidence interval after adjustment for age and sex to die after transplantation for the patients with NCM before transplantation was 0.76 (0.46;1.3).

Table 4 Standardized morbidity ratios adjusted for age, sex and time period for different types of cancer in kidney-transplant recipients restricted to malignancies which developed after transplantation.

Type of cancer (cell type)$	Malignancies Observed*	Malignancies Expected	SMR (95% CI)**	
Head and neck	9	4.18	2.2	(1.1;4.1)
Oral cavity	*5*	*1.33*	*3.8*	*(1.6;9.1)*
Digestive organs	32#	21.28	1.5	(1.1;2.1)
Stomach	*10*	*3.19*	*3.1*	*(1.7;5.8)*
Colon	*12#*	*7.58*	*1.6*	*(0.90;2.8)*
Lower respiratory system	21	16.77	1.3	(0.82;1.9)
Lung	*20*	*16.09*	*1.3*	*(0.85;2.0)*
Bone and soft tissue (sarcoma)	1	1.21	0.83	(0.11;5.9)
Breast	8#	15.02	0.53	(0.26;1.1)
Female genital organs	12	5.01	2.4	(1.4;4.2)
Male genital organs	11	11.35	0.97	(0.54;1.8)
Prostate (1x undefined)	*8*	*10.44*	*0.77*	*(0.38;1.5)*
Urinary tract	15	6.30	2.4	(1.5;4.0)
Kidney	*9*	*2.52*	*3.6*	*(1.9;6.9)*
Urinary bladder	*5*	*3.31*	*1.5*	*(0.63;3.6)*
Central nervous system	2	1.72	1.2	(0.29;4.7)
Endocrine glands	6	0.60	10.0	(4.5;22.3)
Thyroid gland	*5*	*0.52*	*9.5*	*(4.0;22.9)*
Haematolymphopoetic	30	7.41	4.1	(2.8;5.8)
Unknown primary site	5	3.37	1.5	(0.62;3.6)
All first and second non-cutaneous malignancies after transplantation together	152#	94.60	1.6	(1.4;1.9)
Non-melanocytic skin cancer	231	---	---	
Squamous-cell carcinoma	*178*	*4.49*	*39.6*	*(34.2;45.9)*
Basal-cell carcinoma	*138*	---	---	
Other skin cancers	22	---		
Malignant melanoma	*10*	*3.43*	*2.9*	*(1.6;5.4)*
Kaposi sarcoma	*4*	*0.19*	*21.1*	*(7.9;56.1)*
All first and second non-cutaneous malignancies and first cutaneous squamous-cell carcinoma and melanoma together	339	102.45	3.3	(3.0;3.7)

$All malignancies were carcinomas unless otherwise indicated.
* All first and second non-cutaneous malignancies but only the first cutaneous squamous-cell carcinoma after transplantation were counted. Malignancies before transplantation were not counted.
**SMR = standardized morbidity ratio; CI = confidence interval.
#32 malignancies of the digestive organs were observed in 31 patients; 12 colon carcinomas in 11 patients; 8 breast cancers in 7 patients; and 152 non-cutaneous malignancies were observed in 142 patients.

Table 5 Survival rates of the patients after the diagnoses of the most frequently occurring malignancies after kidney transplantation.

Type of cancer (cell type)$	Patients* No.	Deceased N (%)	Survival rate at different time periods after the malignancies (%) 1 year	3 year	5 year	10 year
Head and neck	9	4 (44)	78	67	67	33
Oral cavity	*5*	*2 (40)*	*80*	*80*	*80*	*40*
Digestive organs	31	27 (87)	39	39	22	22
Stomach	*10*	*10 (100)*	*10*	*0*	*0*	*0*
Colon	*11*	*9 (82)*	*64*	*36*	*36*	*36*
Lower respiratory system	21	20 (95)	33	24	18	0
Lungs	*20*	*19 (95)*	*35*	*25*	*19*	*0*
Breast	7	5 (71)	86	38	38	38
Female genital organs	12	7 (58)	92	83	67	48
Male genital organs	11	7 (64)	91	81	81	32
Prostate (1x undefined)	*8*	*5 (63)*	*100*	*86*	*86*	*21*
Urinary tract	14	7 (50)	74	53	53	27
Kidney	*9*	*3 (33)*	*89*	*67*	*67*	*44*
Urinary bladder	*5*	*4 (80)*	*60*	*40*	*40*	*0*
Endocrine glands	6	3 (50)	67	67	67	67
Thyroid gland	*5*	*2 (40)*	*60*	*60*	*60*	*60*
Haematolymphopoetic	30	25 (83)	55	41	33	19
Leukemia	*8*	*8 (100*	*63*	*38*	*38*	*13*
Lymphoma	*22*	*17 (77)*	*52*	*43*	*31*	*23*
All patients who developed non-cutaneous malignancies after transplantation together	142	107 (75)	57	43	36	22
Non-melanocytic skin cancer	231	116 (50)	99	90	77	57
Squamous-cell carcinoma	*178*	*99 (56)*	*97*	*84*	*73*	*56*
Basal-cell carcinoma	*138*	*65 (47)*	*98*	*90*	*77*	*54*
Other skin cancers						
Malignant melanoma	*10*	*7 (70)*	*90*	*60*	*30*	*30*
Kaposi sarcoma	*4*	*3 (75)*	*100*	*100*	*75*	*50*

$All malignancies were carcinomas unless otherwise indicated.
*In the patients with two non-cutaneous malignancies, the date of the last one was used to calculate the survival rate.

Discussion

This long-term cohort study showed that 30 years after transplantation almost 50% of the KTR had developed at least one type of cancer. Cutaneous SCC were, by far, the most frequently occurring cancers, but survival rates of the patients with non-cutaneous malignancies were much lower. Of special interest was the high incidence of thyroid carcinoma which was reported only once before in the literature [14]. The prognosis of the patients with non-cutaneous malignancies before the first transplantation was comparable to the patients without cancer before the transplantation, but it should be noted that these patients had no signs of active malignant disease at the time of transplantation.

Cancers of the oral cavity, stomach, female genital organs, kidney, thyroid gland, as well as leukemias, lymphomas, cutaneous squamous cell carcinoma, Kaposi's sarcoma and malignant melanoma occurred 2 to 40 times more frequently compared with the general population. Common epithelial cancers, such as colon and lung cancer were equally frequently seen in KTR and prostate and breast carcinomas were slightly less commonly seen in KTR compared with the general population. The SMRs of our study are largely in agreement with other studies [2, 3, 14, 19], showing an overall increased risk of malignancies.

Studies in KTR showing survival rates after the diagnosis of cancer are scarce and usually have a shorter follow-up time. Vegso et al showed in Hungary a 5-year survival of 38% after NCM and of 76% after NMSC [6]. A study from Italy showed 1- and 2-year survival rates of 64% and 51% for NCM versus 89% and 89% for NMSC [20]. These data are largely in agreement with the 1-, 3- and 5-year survival rates of 59%, 45% and 39% and 99%, 90% and 77% for NCM and NMSC in our study.

A strength of our study is the long follow-up period with a median follow-up time of more than 9 years and a maximum follow-up of almost 40 years, which is much longer compared with previous studies studying cancer incidence rates [2, 3, 19, 21, 22]. While the long follow-up period is in some ways an advantage, it is also subject to "period effects" i.e. changes in immunosuppressive regimen, cancer screening practice and treatment have all changed dramatically over time. Thus patients with longer follow-up may be less comparable to those with shorter follow-up for reasons other than duration of observation. Another potential weakness of our study is the relatively low power caused by inclusion of patients of a single center so that only the SMRs of the most frequently occurring malignancies could be reliably calculated. In addition, our study was not large enough for a direct comparison of the survival rates of the different malignancies with the non-transplanted population, since the

malignancies were very diverse, both regarding the type of malignancy and the stage of the disease, which factors have an important impact on survival. Finally, medical doctors are following KTR more intensively compared to the general population, which may introduce surveillance bias.

In conclusion, after kidney transplantation, a wide variety of cancers across a large number of organ systems can occur. Many of these cancers occur more frequently than expected based on the occurrence of these cancers in the general population. Because of the high mortality rate of the NCM and the high morbidity rate of cutaneous SCC and BCC, prevention of cancer after transplantation should be a major focus of future research.

Acknowledgements

The authors thank Jan Oosting for providing histopathological examinations and Marko Mallat and Jan Molenaar for providing important clinical data. We are also grateful to Paul Douw van der Krap for his support in laying out the figure.

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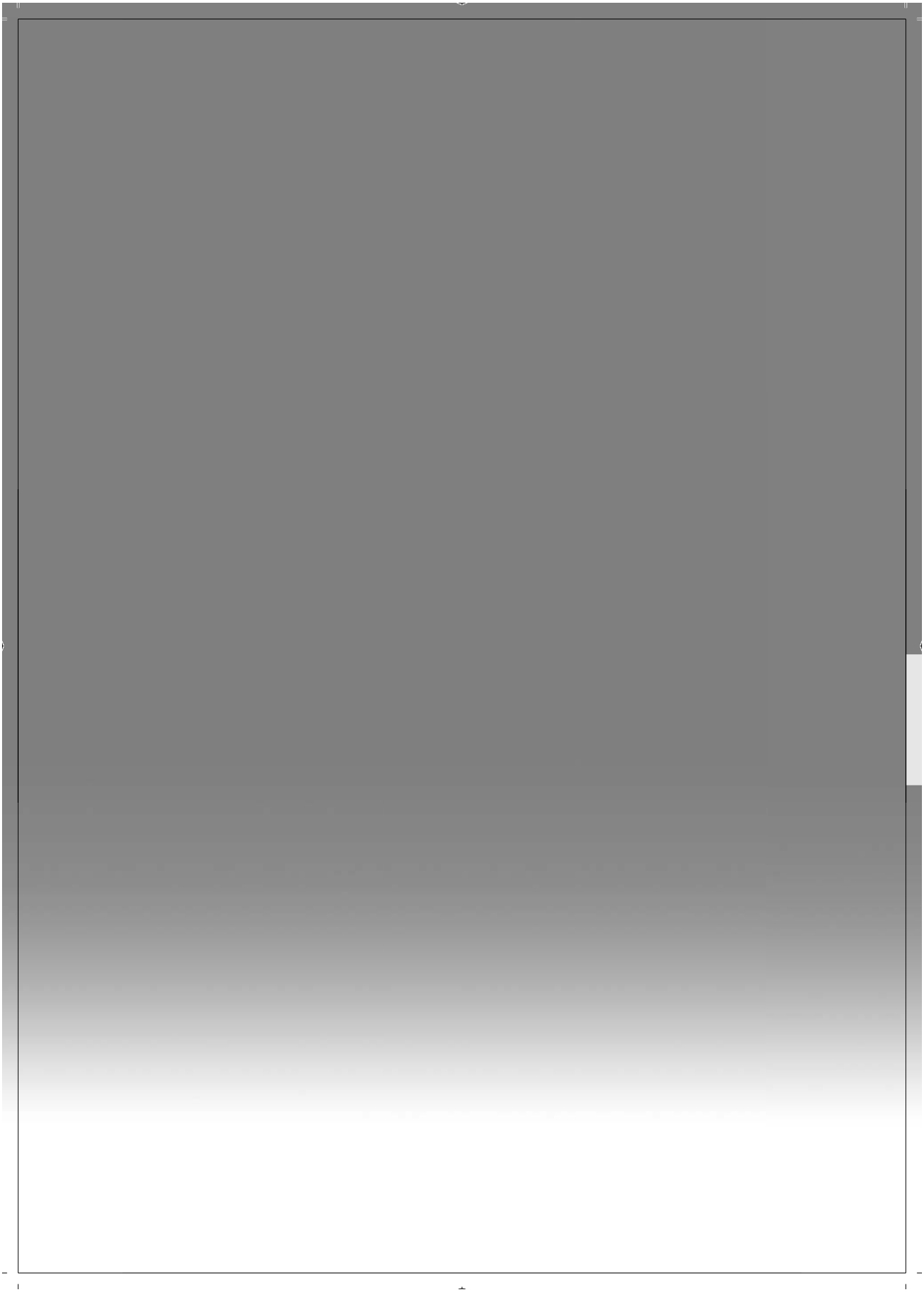

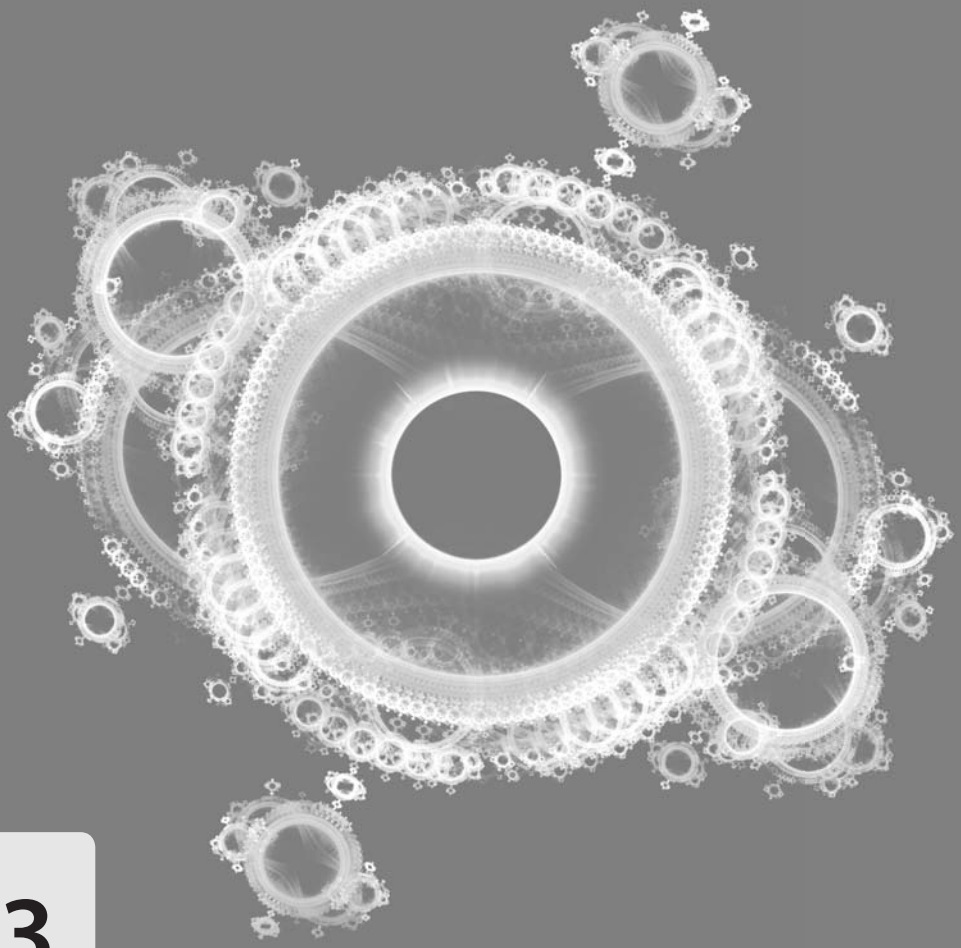

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Subsequent squamous- and basal-cell carcinomas in kidney-transplant recipients after the first skin cancer: cumulative incidence and risk factors

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Transplantation 2010; 27;89 (10):1231-8

Abstract

The risk of skin cancer is highly increased in kidney-transplant recipients, but the risk of subsequent skin cancers is less well studied. The aim of this study was to estimate the cumulative incidence of subsequent squamous- and basal-cell carcinomas and to analyze potential risk factors. All histologically confirmed skin cancers between 1966 and 2006 were included in the study and counted. Cumulative incidences of subsequent squamous- and basal-cell carcinomas were calculated with Kaplan Meier survival analyses. For the analyses of risk factors we used Cox proportional hazard analyses. A total of 239 (13%) out of 1906 kidney-transplant recipients developed skin cancer of whom 222 were diagnosed in our hospital. Altogether 167 (75%) of these 222 patients developed multiple skin cancers. The cumulative incidence of a second skin cancer increased from 32%, 1 year to 59%, 3 years and 72%, 5 years after the first skin cancer. Kidney-transplant recipients who started with squamous-cell carcinoma mainly developed squamous-cell carcinoma and recipients who started with basal-cell carcinoma mainly developed basal-cell carcinoma as second skin cancer. Immunosuppression with azathioprine in combination with prednisone was associated with a significantly increased risk of subsequent squamous-cell carcinomas but not with subsequent basal-cell carcinomas. Skin cancer multiplicity is very common in kidney-transplant recipients. Patients with a first skin cancer are at increased risk for more skin cancers and need to be carefully checked for subsequent skin cancers.

Introduction

The highly increased risk of non-melanocytic skin cancer (NMSC) in organ-transplant recipients (OTR) due to long-term use of immunosuppressive drugs has been frequently reported (1-8). The cumulative incidence of NMSC rises with increasing time after transplantation (1,2,6,9-11). Most studies, however, focused only on the first squamous-cell carcinoma (SCC) or basal-cell carcinoma (BCC). The risk to develop subsequent SCC or BCC in OTR has been scarcely studied (5,11-13). A Scandinavian study showed that 25% of OTR with a first NMSC will develop a second lesion within 13 months, and 50% within 3.5 years (5). A British study reported a mean interval of 15 months between detection of the first and second cancers, and 11 months between the second and third (13). A French study showed that 34% of the 67 heart-transplant recipients and 52% of the 121 kidney-transplant recipients (KTR) with a first SCC developed a subsequent SCC within 3 years after the first SCC. After 5 years these percentages had risen to 64% and 67% in HTR and KTR, respectively (12). A recent study in New Zealand showed that virtually all 96 KTR with skin cancer developed multiple NMSC. After 1 year approximately 30%, after 2 years 50%, after 3 years 60% and after 5 years 80% had developed subsequent NMSC after the first skin cancer (11). Compared with the general population these percentages are very high, since the 3-year cumulative risk of a subsequent SCC after a first SCC in the general population is 18% (14). The risk of a subsequent BCC after the first BCC has not been reported in OTR. In the general population, nearly 50% of patients routinely treated for BCC developed multiple primary BCC during 10 years of observation (15,16). A meta-analysis of 7 independent studies showed a mean 3 year risk of 44% after an initial diagnosis of BCC (14).

The most important factors that favor the development of SCC after transplantation are older age at transplantation, male sex, fair skin type, high cumulative sunlight exposure and length and level of immunosuppression (2,6,8,9,17-21). The risk of BCC is more strongly associated with intermittent, intense sunlight exposure (e.g., sunburn, childhood exposure) (22,23). Other risk factors for BCC are older age at transplantation, fair skin type and immunosuppressive treatment (2,8,21,24-27). Risk factors for subsequent skin cancers are transplantation before 1984, multiple skin cancers at first consultation, hazel-light eye color and advanced age at diagnosis of the first skin cancer (11,12).

Better understanding of the risk of a subsequent SCC and BCC in KTR would help in the design of a more rational follow-up of these patients. The aim of this study was to estimate the cumulative incidence of a second SCC or BCC following the occurrence of the first skin cancer in a cohort of KTR and to study risk factors for the development of subsequent skin cancers.

Materials and methods

Patients

Kidney transplantations have been performed in the Leiden University Medical Centre (LUMC) since 1966. Until December 2006, a total of 1906 KTR received their first transplant.

Most of these patients were regularly followed at the department of Nephrology. Those with cutaneous problems were also seen at the department of Dermatology. At each visit to the skin clinic the entire skin was checked for skin problems. Special attention was focused on the possible presence of keratotic skin lesions and skin cancers. Only few patients were primarily followed by dermatologists in other hospitals than the LUMC.

The following baseline characteristics were recorded for each KTR: sex and dates of birth, transplantation, death, or last follow-up. The risk factors studied in the patients with multiple skin cancers compared to those with a single skin cancer were sex, age, number of years on immunosuppression, country of origin, type of maintenance immunosuppressive therapy, induction and rejection therapy, donor type (living or cadaver), HLA-mismatching and the year the transplantation had been performed. Data about sun exposure and skin type were not collected, because this would have been only possible by questionnaire or physical examination in the living patients. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Data collection: registration of histological diagnoses

All histological diagnoses were systematically computerized at the department of Pathology since 1984 and diagnoses before 1984 were computerized retrospectively starting with the biopsies performed in 1970. In the period between 1966 and 1970 no skin cancers had been diagnosed, which was checked by hand searching in the medical charts of the KTR transplanted before 1970. Dates of the biopsies, locations of the lesions and the histological diagnoses were documented. Routinely, the initial biopsy was followed by an excision and in some cases by a re-excision. In those cases, only the first histologically confirmed diagnosis of this specific skin cancer was taken into account, to prevent double counting. In-situ carcinomas (Bowen's disease), precursor lesions (actinic keratoses and keratoacanthomas) and skin cancers other than squamous-cell and basal-cell carcinoma (e.g. malignant melanoma, Kaposi sarcoma and adnex carcinomas) were not considered in this study.

The country of origin was used as a rough estimation of skin type. Information about the initial and maintenance immunosuppressive therapy of the patients with

one and more skin cancers was obtained from the Eurotransplant database. Type of induction therapy and the number and type of rejection treatments were collected from the flow sheets in the medical charts of the department of Nephrology. For the KTR the immunosuppressive treatment initially consisted of duo therapy with prednisolone (P) and azathioprine (Aza), but shortly after 1986 all new KTR were immunosuppressed with P and cyclosporine A (CsA). After 1996 triple therapy became the treatment of choice where, initially, most new KTR were treated with P, mycofenolatemofetil (MMF) and CsA and later most new KTR were treated with P, MMF and tacrolimus (Tac).

KTR, in whom acute graft rejections were observed, were almost always initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection a second and third rejection treatment with anti-thymocyte globulin (ATG) and once more methylprednisolone, respectively, were given. In exceptional cases muronomab-CD3 (OKT3) was given when a fourth rejection treatment was needed. With the exception of some rare patients, induction treatments with ATG and/or OKT3 were not given to KTR who were transplanted in the LUMC. Starting in 2000, however, induction treatment with basiliximab became common practice.
The degree of HLA-mismatching for HLA-A, HLA-B and HLA-DR was assessed by counting the antigens present in the donor but absent in the recipient.

Statistical analyses

For the characterization of the patients with and without skin cancer we used the data of all 1906 patients who were transplanted in Leiden between 1966 and 2006. Kaplan Meier survival analyses were used to estimate the cumulative incidences of skin cancer after transplantation. As opening dates for these analyses we used the date of the first transplantation; as closing dates we used the date of diagnosis of the first skin cancer, the date of the patient's death or the date of last follow up. Skin cancers before transplantation were not considered in these analyses. Patients were not censored from the analyses at graft failure. Differences between patients with and without skin cancer were analyzed by Chi-square for categorical variables and Student's T-tests for continuous variables.

For the analyses of subsequent skin cancers in patients with skin cancer we used the 222 patients with NMSC who were diagnosed at the LUMC. Kaplan Meier survival analyses were used to estimate the cumulative incidence of subsequent skin cancers in patients with a prior skin cancer. Cox proportional hazard analyses were used to identify potential risk factors of NMSC multiplicity. As opening dates for both analyses we used the date of the first skin cancer; as closing dates we used the date of diagnosis

of the subsequent SCC or BCC, the date of the patient's death, the date of last follow up, the date that they were lost to follow-up, or we used the date of the end of the study (June 1, 2007). Some patients had two NMSC at the time that they presented themselves with the first NMSC. In these patients the third NMSC was counted as the second presentation of NMSC.

The initial and maintenance immunosuppressive therapies were categorized into three basic treatment groups: duo or triple therapy with Aza in any combination; duo or triple therapy with MMF in any combination; and duo therapy without Aza or MMF (i.e. a combination of prednisolone with CsA). If no data were available for the maintenance immunosuppressive therapy the data of the initial immunosuppressive therapy were used. For all our analyses with immunosuppressive therapy, we used the sub-categorization of the maintenance therapy because the patients were, generally, most prolonged exposed to this regimen.

Because ATG and OKT3 exert by far the highest immunosuppressive effect, induction and rejection treatments were dichotomized into those with and without ATG and/or OKT3. Because the biological effects of ATG and OKT3 are supposed to be similar before and after the transplantation, exposures to ATG and/or OKT3 as induction or rejection treatment were also combined for our analyses.

Statistical analyses were performed with SPSS software (version 16.0, SPSS, Chicago, IL).

Results

Baseline characteristics

Altogether 239 (12.5%) of the 1906 KTR transplanted between 1966 and 2006 had developed at least one NMSC. The baseline characteristics of the patients with and without NMSC, SCC or BCC are depicted in Table 1. Eight out of the 239 patients (3.3%) had developed the first skin cancer before transplantation (2 both SCC and BCC, 6 only BCC), with a median time of 1.3 years before transplantation (Table 1). The majority of the patients with NMSC were transplanted before 1986, whereas patients without skin cancer were more frequently transplanted after 1986 (Table 1, $P<0.001$). The follow-up period of the KTR with skin cancer was, therefore, much longer ($p = 0.001$). The sex distribution did not differ between patients with and without skin cancer ($p = 0.218$) (Table 1). In the Cox proportional hazard model older age at transplantation was associated with a significantly increased risk of skin cancer (3.6% [95% CI 2.6%;4.7%] increase for each additional year of age, $P < 0.001$). After stratification for time period after transplantation this association was also clear in Table 1. The median age at

Table 1 Characteristics of kidney-transplant recipients with and without skin cancer.

	No skin cancer	NMSC	SCC*	BCC*
Number of patients	**1667**	**239**	**180**	**146**
Year of transplantation: N (%)				
1966-1975	179 (10.7)	62 (25.9)	54 (30.0)	32 (21.9)
1976-1985	359 (21.5)	105 (43.9)	85 (47.2)	66 (45.2)
1986-1995	486 (29.2)	55 (23.0)	35 (19.4)	33 (22.6)
1996-2005	643 (38.6)	17 (7.1)	6 (3.3)	15 (10.3)
No. of male: N (%)	1019 (61.1)	156 (65.3)	121 (67.2)	96 (65.8)
Median age at transplantation (years) (25%-75%)	44.2 (32.8 – 55.0)	41.6 (29.6 – 49.4)	39.3 (29.1 – 47.5)	42.2 (31.8 – 50.7)
Median follow up time after KT (years) (25%-75%)	7.8 (3.2 - 27.3)	20.6 (13.1 – 27.3)	22.1 (14.6 – 27.8)	19.9 (12.2 – 27.3)
Median age at transplantation in years (N) stratified for follow-up period after KT				
0-1	49.5 (271)	64.9 (3)	65.1 (2)	64.9 (3)
2-7	50.1 (574)	59.4 (19)	62.5 (11)	59.1 (12)
8-12	46.5 (299)	52.7 (37)	53.2 (19)	52.7 (27)
13-17	40.4 (226)	46.0 (42)	46.2 (34)	44.6 (22)
18-22	33.1 (135)	39.3 (36)	38.6 (30)	39.3 (19)
23+	25.9 (162)	30.8 (102)	30.5 (84)	33.8 (63)
Median time to first skin cancer (years) (25%-75%)		11.4 (6.9 – 17.4)	12.6 (8.0 – 18.5)	12.1 (6.6 – 17.2)
Median age at first skin cancer (years) (25%-75%)	--	53.3 (44.4 – 59.9)	54.1 (44.7 – 60.2)	53.6 (46.7 – 60.0)

NMSC, non-melanocytic skin cancer; SCC, squamous-cell carcinoma; BCC, basal-cell carcinoma; KT, kidney transplantation.
*Some patients had both SCC and BCC. This fact is reflected here by overlapping of the numbers of patients in these categories.

which the KTR developed their first NMSC was 53.3 years. There were 9 patients who died of metastases of SCC (5 times the SCC was located on the arm or shoulder and 4 times the location was in the face, on the ear, or on the skull). None of the patients died of BCC.

The cumulative incidence of NMSC after transplantation was 9% after 10 years, 24% after 20 years and 40% after 30 years. For SCC this incidence was 6%, 19%, and 33% and for BCC 5%, 14% and 24% after these time periods, respectively. This is graphically presented in the supplementary Figure.

Subsequent NMSC

Eighty-six out of the 222 KTR who had histologically confirmed skin cancers at the LUMC had developed only SCC, 53 only BCC and 83 KTR had developed both SCC and BCC during the follow-up time. More than 75% KTR had developed subsequent skin cancers. Twenty-four patients presented with 2 or more NMSC at the first presentation of whom 6 patients had 3 NMSC. Approximately 60% developed a third and almost 50% a fourth NMSC. A total of 25 patients (11.3%) developed more than 20 histologically confirmed NMSC. The exact numbers of skin cancer are presented in the supplementary Table. The median number of NMSC per KTR was 3.0 with a mean of 8.3 (min-max 1-76), for SCC this was 3.5 and 8.1 (min-max 1-68), and for BCC 2.0 and 3.4 (min-max 1-28), respectively. Multiplicity was more evident for SCC than for BCC.

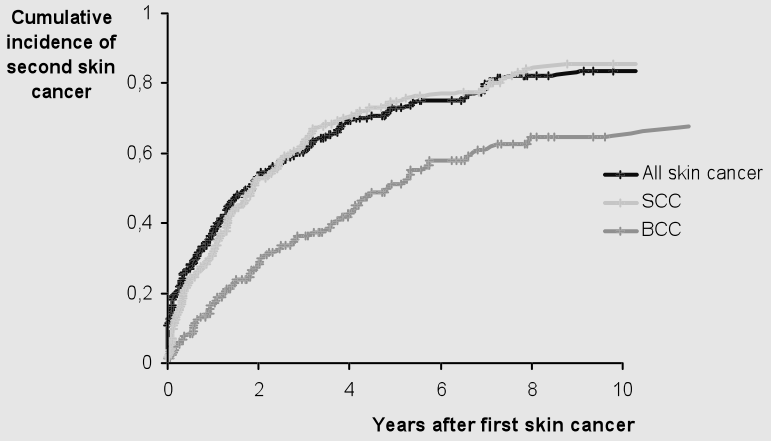
The cumulative incidence of a second NMSC after the first NMSC increased from 32% after 1 year to 59% after 3 years and 72% after 5 years (Figure 1). The cumulative incidences for a second SCC were 31%, 62% and 75%, respectively. The cumulative incidences for a second BCC were lower and were 16%, 37% and 51% after these time periods, respectively (Figure 1).

The distribution of SCC and BCC on the body is presented in Table 2. When patients developed only one SCC this tumor was more frequently located on the head and neck, whereas subsequent SCC were more common on the hands and fingers. Approximately 50% of the BCC were located on the head and neck in both patients with only one BCC as patients with subsequent BCC. Patients with one BCC developed the tumor more frequently on the hands and fingers and patients with multiple BCC developed them more frequently on the trunk (Table 2).

The risk of SCC and BCC in KTR with SCC or BCC as first NMSC

A total of 127 KTR started with SCC as the first skin cancer. Hundred (78.7%) of them developed a second SCC and 41 (32.3%) developed a first BCC at a later time point. Altogether 95 KTR started with BCC as the first skin cancer of whom 54 (56.8%) developed a second BCC and 41 (43.2%) a first SCC at a later time point. We asked the question whether it would be relevant for the risk and type of subsequent skin cancers if the first skin cancer would be SCC or BCC.

Figure 1 Cumulative incidence of second non-melanocytic skin cancer (NMSC), squamous-cell carcinoma (SCC) and basal-cell carcinoma (BCC) in respectively 222, 178 and 138 kidney-transplant recipients (KTR). The numbers of KTR at risk in relation to the years after transplantation are indicated in the table below the figure.



Numbers of KTR at risk at the specified time periods after transplantation (yrs)

Type of skin cancer	0	1	3	5	10
All skin cancer	222	143	76	44	12
SCC	169	111	53	30	11
BCC	136	103	65	39	12

Table 2 Location of tumors by one or multiple skin cancer.

	One SCC	Two and more SCC	p-value	One BCC	Two and more BCC	p-value
Number of tumors	**38**	**1338**		**60**	**482**	
Location tumors (%)						
Head and neck	19 (50.0)	404 (30.2)	<0.001	30 (50.0)	232 (48.1)	<0.001
Trunk	5 (13.2)	74 (5.5)		3 (5.0)	130 (27.0)	
Arms	6 (15.8)	131 (9.8)		7 (11.7)	56 (11.6)	
Hands and fingers	4 (10.5)	463 (34.6)		11 (18.3)	16 (3.3)	
Legs and feet	1 (2.6)	211 (15.8)		3 (5.0)	30 (6.2)	
Other sites	0	4 (0.3)		0	0	
Unknown	3 (7.9)	51 (3.8)		6 (10.0)	18 (3.7)	

SCC, squamous-cell carcinoma; BCC, basal-cell carcinoma

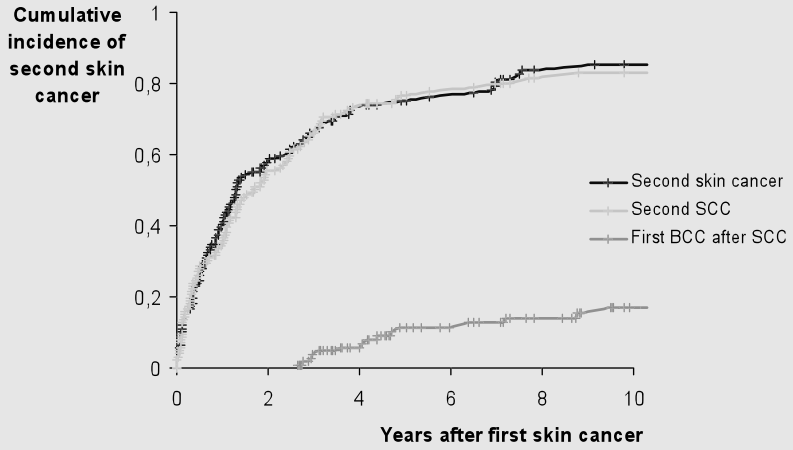
Figure 2A shows that KTR who started with SCC mainly developed SCC as second skin cancer and Figure 2B shows that KTR who started with BCC mainly developed BCC as second skin cancer. After the development of the first SCC in the latter group of patients, the risk of subsequent SCC was similar to the risk of subsequent SCC in the KTR who started with SCC.

Risk factors for subsequent skin cancers

The distribution of potential risk factors in the patients with one and those with subsequent SCC or BCC and the results of univariate analyses are presented in Table 3. Multivariate analyses are presented in Table 4. In both types of analyses maintenance immunosuppressive therapy with Aza was associated with the highest risk of subsequent SCC, but not of subsequent BCC. Patients who were immunosuppressed with CsA had a significantly reduced risk of subsequent SCC compared to patients who were immunosuppressed with Aza (Tables 3 and 4). A potentially decreased risk of MMF could not be assessed, because of insufficient patients in this treatment category.

In the univariate analyses the time period of transplantation between 1986 and 1995 was associated with a reduced risk of subsequent SCC (Table 3), but this reduced risk disappeared in the multivariate analyses (Table 4). In contrast, the risk of subsequent BCC tended to increase in the later time periods, although significance was not reached (Table 4).

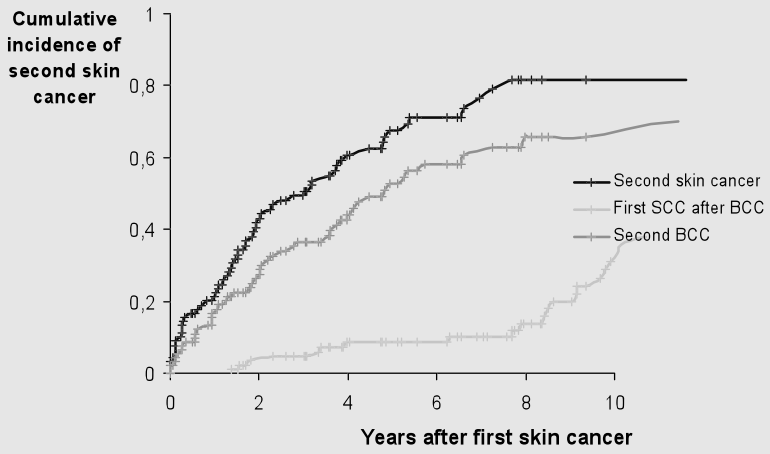
Figure 2 **A**. Cumulative incidence of a second non-melanocytic skin cancer (NMSC), squamous-cell carcinoma (SCC) and basal-cell carcinoma (BCC) in patients with a SCC as first event. The numbers of kidney-transplant recipients (KTR) at risk in relation to the years after transplantation are indicated in the table below the figure.



	Numbers of KTR at risk at the specified time periods after transplantation (yrs)				
Type of skin cancer	0	1	3	5	10
All skin cancer	127	75	38	25	10
SCC	127	81	39	21	10
BCC	127	107	100	75	48

Age at transplantation, which is a risk factor for the first skin cancer, was not a statistically significant risk factor for subsequent SCC or BCC (Table 3), although there was a trend of an association between older age and skin cancer in the multivariate analyses (Table 4). Patients with a longer time between the transplantation and the development of the first SCC had an increased risk of developing subsequent SCC, but the time between transplantation and the first BCC did not influence the development of subsequent BCC (Tables 3 and 4).

Figure 2 B. Cumulative incidence of a second non-melanocytic skin cancer (NMSC), squamous-cell carcinoma (SCC) and basal-cell carcinoma (BCC) in patients with a BCC as first event. The numbers of kidney-transplant recipients (KTR) at risk in relation to the years after transplantation are indicated in the table below the figure.



	Numbers of KTR at risk at the specified time periods after transplantation (yrs)				
Type of skin cancer	0	1	3	5	10
All skin cancer	95	68	38	19	2
SCC	95	87	78	64	30
BCC	95	73	47	27	8

Patients who were transplanted with a kidney of a living donor had an increased risk of subsequent BCC compared to patients who received a cadaver kidney (Table 4). Donor type did not affect the risk of subsequent SCC.

Table 3 Risk factors for subsequent squamous-cell carcinomas or basal-cell carcinomas, univariate analyses.

	One SCC	Two or more SCC	Hazard ratio	95% CI	One BCC	Two or more BCC	Hazard ratio	95% CI
	N (%)	N (%)			N (%)	N (%)		
Number of patients	**39**	**129**			**60**	**76**		
Sex								
Female	11 (28.2)	47 (36.4)	1		24 (40.0)	24 (31.6)	1	
Male	28 (71.8)	82 (63.6)	0.97	0.68-1.4	36 (60.0)	52 (68.4)	1.4	0.86-2.4
Donor type								
Living	1 (2.8)	11 (8.9)	1		4 (7.0)	11 (15.1)	1.0	
Cadaver	35 (97.2)	112 (91.1)	0.86	0.46-1.6	53 (93.0)	62 (84.9)	0.72	0.38-1.4
Period of transplantation								
1966-1975	9 (23.1)	43 (33.3)	1		15 (25.0)	16 (21.1)	1	
1976-1985	16 (41.0)	65 (50.4)	0.87	0.59-1.3	21 (35.0)	40 (52.6)	1.5	0.81-2.7
1986-1995	13 (33.3)	19 (14.7)	*0.56*	*0.32-0.97*	14 (23.3)	16 (21.1)	1.2	0.59-2.6
1996-2006	1 (2.6)	2 (1.6)	---*	---	10 (16.7)	4 (5.3)	0.73	0.21-2.5
Age at transplantation in years								
0-49	26 (66.7)	109 (84.5)	1		43 (71.7)	59 (77.6)	1	
50-59	7 (17.9)	12 (9.3)	0.68	0.38-1.2	9 (15.0)	9 (11.8)	0.81	0.37-1.8
60 or more	6 (15.4)	8 (6.2)	0.69	0.33-1.4	8 (13.3)		1.4	0.62-3.1

Table 3 Risk factors for subsequent squamous-cell carcinomas or basal-cell carcinomas, univariate analyses, continued.

	One SCC	Two or more SCC	Hazard ratio	95% CI	One BCC	Two or more BCC	Hazard ratio	95% CI
	N (%)	N (%)			N (%)	N (%)		
Number of patients	**39**	**129**			**60**	**76**		
Time to first skin cancer in years								
0-7	18 (46.2)	36 (27.9)	1		23 (38.4)	33 (43.4)	1	
8-12	9 (23.1)	34 (26.3)	1.6	0.94-2.6	17 (28.3)	17 (22.4)	0.78	0.38-1.6
13-17	5 (12.8)	26 (20.2)	2.1	1.2-3.6	9 (15.0)	17 (21.1)	0.99	0.54-1.8
18 or more	7 (17.9)	44 (25.6)	1.9	1.2-3.2	11 (18.3)	10 (13.2)	1.0	0.53-1.9
ATG or OKT3 as induction or rejection treatment								
No	36 (92.3)	125 (96.9)	1		53 (88.3)	72 (94.7)	1	
Yes	3 (7.7)	4 (3.1)	0.74	0.27-2.0	7 (11.7)	4 (5.3)	0.41	0.13-1.3
Type of maintenance immunosuppression								
Aza in any combination#	23 (59.0)	107 (82.9)	1.0		34 (56.7)	51 (67.1)	1.0	
MMF in any combination##	0 (0.0)	6 (4.7)	---*	---	9 (15.0)	4 (5.3)	0.62	0.23-1.7
CsA without Aza or MMF###	16 (41.0)	16 (12.4)	*0.43*	*0.25-0.73*	17 (28.3)	21 (27.6)	1.1	0.61-1.8

ATG, anti-thymocyte globulin; Aza, azathioprine; BCC, basal cell carcinoma; CsA, cyclosporine A; MMF, mycofenolatemofetil; P, prednisolone; SCC, squamous cell carcinoma; Tac, tacrolimus; OKT3, muronomab-CD3; CI, confidence interval.
#Duo-therapy with P and Aza was given to all patients with the exception of one patient with BCC who was treated with P, Aza and CsA.
##Duo-therapy with P and MMF was given to 5 patients, triple therapy with P, MMF and CsA to 7 patients and with P, MMF and Tac to 3 patients.
###Duo-therapy with P and CsA was given to all patients in this category.
* When there were less than 7 patients in both categories together, the hazard ratio was not calculated. Statistically significant hazard ratios are indicated in *italic*.

Table 4 Risk of multiple SCC or BCC in kidney transplant recipients with adjustments for potentially confounding factors, multivariate analyses.

	SCC		BCC	
	Adjusted* hazard ratio	**95% CI**	**Adjusted* hazard ratio**	**95% CI**
Sex				
Female	1		1	
Male	1.2	0.79-1.7	1.5	0.87-2.6
Donor type (%)				
Living	1.0		1.0	
Cadaver	1.1	0.56-2.1	*0.41*	*0.19-0.90*
Period of transplantation				
1966-1975	1		1	
1976-1985	0.97	0.64-1.5	1.7	0.88-3.2
1986-1995	0.91	0.39-2.1	1.8	0.65-5.3
1996-2006	---**	---	0.89	0.18-4.5
Age at transplantation in years				
0-49	1		1	
50-59	1.6	0.74-3.3	1.1	0.40-3.3
60 or more	1.8	0.68-5.0	1.8	0.65-5.0
Time to first skin cancer in years				
0-7	1		1	
8-12	1.6	0.89-3.0	0.85	0.38-1.9
13-17	2.0	1.1-3.9	0.91	0.43-1.9
18 or more	1.8	0.94-3.5	1.2	0.53-2.7
ATG or OKT3 as induction or rejection treatment				
No	1		1	
Yes	1.3	0.38-4.1	0.40	0.11-1.5
Type of maintenance immunosuppression				
Aza in any combination	1		1	
MMF in any combination	---	---	0.83	0.23-3.0
CsA without Aza or MMF	*0.41*	*0.20-0.84*	0.80	0.33-1.9

ATG, anti-thymocyte globulin; Aza, azathioprine; BCC, basal cell carcinoma; CsA, cyclosporine A; MMF, mycofenolatemofetil; P, prednisolone; SCC, squamous cell carcinoma; Tac, tacrolimus; OKT3, muronomab-CD3; CI, confidence interval.
* Adjusted for age at transplantation, sex, donor type, year of transplantation, time to first skin cancer, ATG or OKT3 as induction or rejection treatment and maintenance immunosuppression.
** When there were less than 7 patients in both categories together, the hazard ratio was not calculated.
Statistically significant hazard ratios are indicated in *italic*.

Male sex and ATG or OKT3 as induction or rejection therapy were not significantly associated with an increased risk of subsequent SCC or BCC (Tables 3 and 4). HLA-mismatching also did not influence the risk of subsequent SCC or BCC (data not shown). Country of origin was not analyzed as a risk factor, since all patients with SCC originated from the Netherlands and only two of the patients with BCC originated from another country, namely China and Turkey, respectively.

Discussion

This study confirms the high risk of subsequent NMSC in KTR who have developed a first NMSC. This risk was especially high for SCC but was also substantial for BCC. The tumor burden was very high in these patients with 24% of KTR developing 10 or more skin cancers within a short time period. The high risk of subsequent SCC was only apparent after the appearance of a first SCC: the risk of SCC was much lower in the KTR who presented themselves with BCC, but after the appearance of the first SCC subsequent SCC appeared equally fast in the latter group.

The 3-year risks of 59% of NMSC and 62% of subsequent SCC in our study are comparable with the risks of 60% of NMSC in New Zealand (11) and 52% of SCC in France (12) and the 5 year-risks of 72% of NMSC and 75% of SCC in our study are comparable with the risks of 80% of NMSC in New Zealand (11) and 67% of SCC in France (12). The risk of subsequent SCC in KTR is much higher compared with the general population, in which a 3-year cumulative risk of subsequent SCC of only 18% was calculated (14).

There are no previous studies describing the risk of subsequent BCC in KTR. The 3-year risk of 37% and the 5 year-risk of 51% of subsequent BCC in our study are comparable with previous observations in the general population where a meta-analysis of 7 studies between 1972 and 1993 showed a 3-year cumulative risk of 44% (14). A more recent study from Queensland, Australia showed that 43% of people with BCC developed a second BCC within 4.5 years (15).

KTR who started with SCC mainly developed SCC as subsequent skin cancers and recipients who started with BCC mainly developed BCC. This could possibly be explained by different lifestyle factors of the patients. The risk of SCC is considered to be associated with chronic cumulative sun exposure whereas BCC is more associated with intermittent, intense sun exposure (28). Alternatively, a state of immune unresponsiveness may have been induced by the occurrence of the first skin cancer with immunologic tolerance to subsequent skin cancers with the same antigenic profile as the possible result.

The type of maintenance immunosuppressive therapy was the most important risk factor of subsequent SCC. Patients who were immunosuppressed with Aza had an almost three times increased risk of subsequent SCC compared to patients who were immunosuppressed with CsA. The period of transplantation between 1986 and 1995 was associated with a decreased risk of SCC in the univariate analyses. This period coincided with the start of immunosuppression with CsA. In the multivariate analyses this risk disappeared and only the association between immunosuppressive regimen and SCC remained. These analyses suggest that the more frequent immunosuppression with CsA between 1986 and 1995 may explain the less increased risk of SCC in this time period. The type of immunosuppression was not significantly associated with subsequent SCC in other studies (11,12), but there are several reports that patients who are immunosuppressed with Aza have an increased risk of a first SCC compared to patients who are immunosuppressed with other drugs (10,29). Aza has been recognized to increase photosensitivity of the skin and also enables UVA to directly damage DNA (30). These characteristics of Aza may increase the risk of both first and subsequent SCC in patients who are chronically using this drug.

Longer time periods between transplantation and the development of the first SCC were associated with an increased risk of subsequent SCC. Apparently, the duration of the immunosuppressive therapy influences the development of subsequent SCC after the first SCC. The induction period of the first BCC did not influence the development of subsequent BCC.

Male sex has been reported as a risk factor for multiple skin cancers (12,16), but we only observed a trend of an increased risk of multiple BCC for male patients. A kidney of a living donor compared with a cadaver kidney was associated with an increased risk of subsequent BCC but not of subsequent SCC. Additional studies are needed to confirm this association.

Sun exposure has been reported as an important risk factor for multiple lesions (21,31) and Euvrard et al showed that light color of the hair and eyes and fair skin type were predictive of multiple SCC (12). In our study we were not able to assess these risk factors.

In conclusion, this study confirmed and consolidate that skin cancer multiplicity, in particular of SCC, is very common in KTR. Transplant physicians should be aware of this problem and easy accessible reference of KTR to the dermatologist should be accomplished. KTR with a first skin cancer should be carefully checked for subsequent skin cancers, preferably at dermatology departments with specialized skin cancer care.

Acknowledgements

The authors would like to thank Jan Molenaar and Koos Mistrate Haarhuis for providing important clinical data. We are also grateful to Paul Douw van der Krap for his support in lay outing of the figures.

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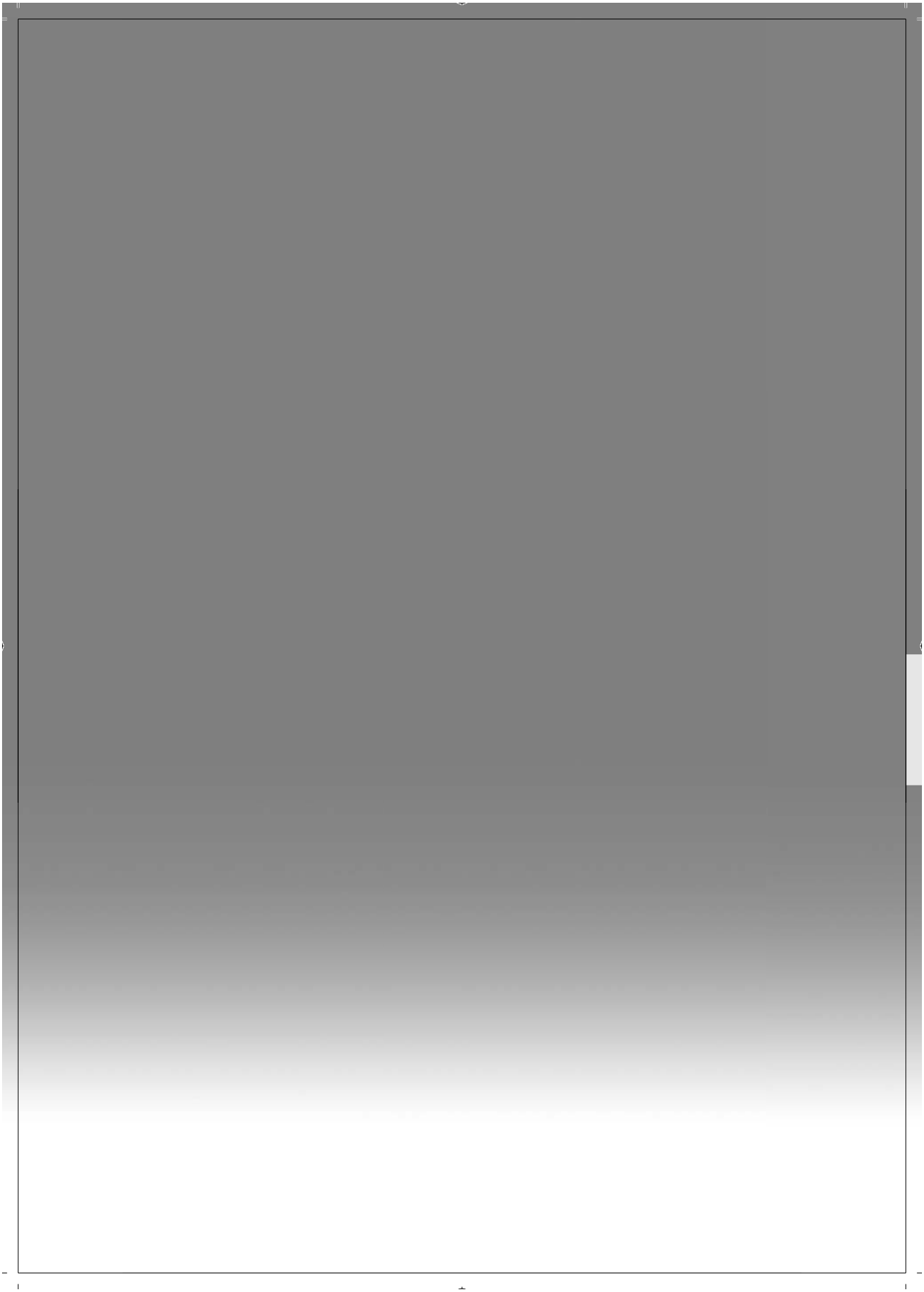

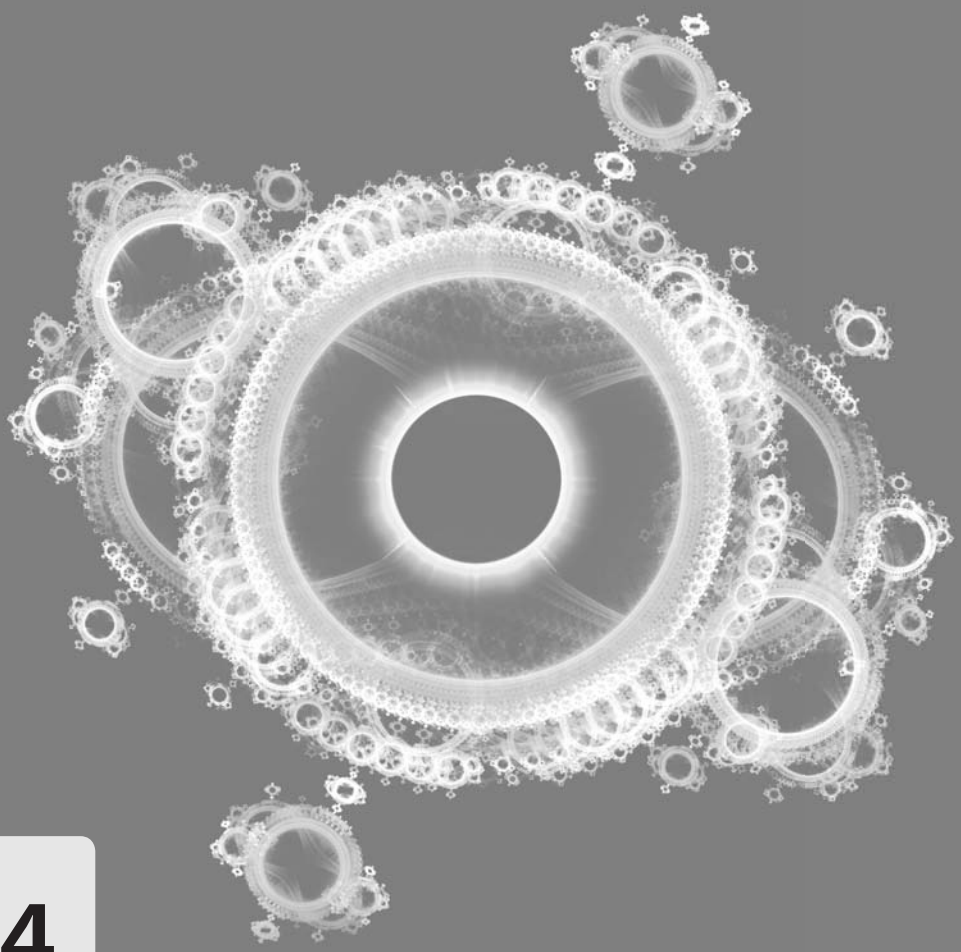

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Trends of skin diseases in organ-transplant recipients transplanted between 1966 and 2006: a cohort study with follow-up between 1994 and 2006

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British Journal of Dermatology 2010;162(2):390-6

Abstract

Skin diseases are frequently observed in organ-transplant recipients. To count the registered skin diseases in all 2136 organ transplant recipients who had been transplanted in a single centre between 1966 and 2006 and to calculate their relative contribution in relation to the number of years after transplantation. All registered skin diseases which were entered into a computerized system between 1994 and 2006 at the Leiden University Medical Centre were counted and their relative contributions were calculated. Between 1994 and 2006, 2408 skin diseases were registered in 801 of 1768 organ-transplant recipients who were at risk during this specific time period. The most commonly recorded diagnoses were skin infections (24.0%) followed by benign skin tumours (23.3%) and malignant skin lesions (18.2%). The relative contributions of infectious and inflammatory disorders decreased with time after transplantation, whereas the contribution of squamous cell carcinomas strongly increased with time. This study gives a systematic overview of the high burden of skin diseases in organ-transplant recipients. The relative distributions of skin diseases importantly changed with time after transplantation, with squamous cell carcinoma contributing most to the increasing burden of skin diseases with increasing time after transplantation.

Introduction

As a consequence of long-term immunosuppressive therapy cutaneous side effects are frequently observed in organ-transplant recipients (OTR) [1-7]. Common skin lesions in OTRs are viral, fungal and bacterial infections [8-10], and benign, premalignant and malignant skin tumours [2-4;6]. The most prevalent types of skin cancer in OTRs are squamous cell carcinomas (SCC) followed by basal cell carcinomas (BCC) [2;3;11-14].

The prevalence of skin infections is very high and several studies have described that 55-97% of OTRs have some type of infection [9;10;15-17]. The spectrum of skin infections differs according to the post-transplant time period [10]. During the first post-transplant month infections result mainly from surgical interventions [8]. After the first post-transplant month, the nature of infectious skin diseases is more frequently a result of severe immunosuppression, manifesting in infections with herpes viruses (herpes simplex virus, varicella zoster virus, cytomegalovirus, Epstein-Barr virus), yeasts (Candida) and bacteria [10]. Six months and more after transplantation the chronic and progressive infections start to exert clinically significant effects [8;10], of which infections with human papillomaviruses (HPVs) have been most frequently described [8;18;19].

The highly increased risks of SCCs and BCCs in OTRs have been frequently described [2-4;6]. The cumulative incidences of these tumours rise with increasing time after transplantation. In highly sun-exposed areas such as Australia the cumulative incidence has been reported to be 70% after 20 years [2], whereas in more temperate climates, for instance the United Kingdom, the Netherlands, Ireland and Norway, cumulative incidences between 20 and 40% after 20 years have been reported [3;4;6;20]. Compared with the large number of studies focusing on the development of skin tumours in OTRs, infectious and inflammatory skin diseases have been studied less frequently [8-10;15-19].

The first aim of this study was to estimate the frequency of registered skin diseases diagnosed in a single centre between 1994 and 2006. The second aim was to calculate the relative contributions of the different skin diseases in relation to the number of years after transplantation.

Patients and methods

Patients

All 2136 patients who had received a first kidney (n = 1910) or a simultaneous pancreas and kidney transplantation (n = 226) between March 1966 and December 2006 at the

Leiden University Medical Centre (LUMC) were included in this cohort study. The follow-up period of these patients started in 1994, at the time that computerization of the registered skin diseases had started, and ended in December 2006. A total of 347 patients had died and 21 patients were lost to follow-up before 1994, resulting in 1225 patients who were at risk at the start of the follow-up period in 1994. Including the patients who were transplanted between 1994 and 2006, 1768 patients were at risk during the 13-year follow-up period.

Most of these patients were regularly seen at the Department of Nephrology. Those with cutaneous problems were also seen at the Department of Dermatology and, since 1996, these patients were concentrated in a specialized OTR skin clinic located at the Department of Dermatology of the LUMC.

At each visit to the skin clinic the entire skin was checked for skin problems. Special attention was focused on the possible presence of keratotic skin lesions and skin cancers. Only few patients were monitored by dermatologists in hospitals other than the LUMC. Cutaneous diagnoses in clinics outside the LUMC were not considered in this study.

The following baseline characteristics were recorded for every OTR: date of birth, sex, date of transplantation and date of death or last follow-up. Permission for the study was granted by the Medical Ethical Committee of the LUMC.

Data collection

To identify the registered skin diseases we used several computerized LUMC diagnostic registration systems. Starting in 1994 the outpatient clinical information was registered with a medical registration program (MEDREG), and inpatient clinical information was registered in a National Medical Registration database. In 2003 "diagnosis treatment combinations" (DBCs) were introduced in The Netherlands for the registration and reimbursement of hospital and medical specialist care and the use of MEDREG was abandoned. Between 2003 and 2005 multiple diagnoses per patient per year still could be introduced into the DBC system. Since 2005, the DBC system was also used for reimbursement of costs by the health insurance, limiting the registration of skin diagnoses to one per patient per year. Usually the presenting diagnosis was registered and, if there were multiple diagnoses, the most severe diagnosis was registered. Registration was based on ICD9 and ICD10 codes allowing the registration of most dermatological diagnoses. Follow-up data were used for the period between 1 January 1994 when computerisation had started and 31 December 2006, the arbitrary end of this study. The LUMC diagnostic registration system has the advantage that it also diagnoses skin diseases without histological confirmation, but the

disadvantage that data collection was not always complete. Owing to the DBC registration system, registration of skin diagnoses was limited to one diagnosis per patient from 2005 onwards.

The skin diseases were categorized into two main groups: A, skin diseases other than tumours and B, skin tumours. Group A was subdivided into (i) skin infections; (ii) inflammatory skin conditions; (iii) vascular skin problems; (iv) wounds and (v) remainder. Group B was subdivided into (i) benign skin tumours; (ii) premalignant skin tumours and (iii) malignant skin tumours. We arbitrarily categorized verrucae, condylomata and mollusca contagiosa as benign skin tumours because of their clinical appearance, although they are caused by members of the papillomavirus and poxvirus families, respectively. We categorized keratoacanthoma as a pre-malignant skin lesion instead of a benign skin lesion, because in the OTR population keratoacanthomas are often difficult to distinguish from SCC.

Statistical analyses

The follow-up time for each patient was computed as the number of years between the first transplantation and the end of the study. For the end of the study we used the date of the patient's death, the date of the last follow up or the arbitrary end of the study on 31 December 2006. The follow-up years were categorized into categories of 5 years ranging from 0-4 years up to 35-39 years after transplantation. The numbers of patients at risk were calculated for each follow-up category.

Results

Registered skin diseases between 1994 and 2006

The number of patients with registered skin diseases and the total number of registered skin diseases which were entered into the computerized system between 1994 and 2006 in OTRs who were transplanted after 1966 and still alive in 1994 are presented in Table 1. Altogether 2408 skin diseases were registered in 801 (45.3%) of 1768 patients who were at risk during this 13-year period, corresponding with a mean number of 3.0 skin diseases per patient. The greatest number of skin diseases per patient in this period was 34.

The 2408 registered skin diseases were equally distributed among skin tumours (1274) and other skin diseases (1134) (Table 1). The diagnoses of skin tumours tended to concentrate in fewer patients (456 patients) than the other skin diseases (591 patients). Skin diseases other than tumours were diagnosed almost 10 years earlier

Table 1 Distribution of skin diseases between 1994 and 2006 in 591 out of 1768 organ transplant recipients who were transplanted after 1966 and were still alife in 1994.

Skin diseases	Number of patients	Number of diseases	Years after transplantation		
			Median	25 percent	75 percent
All skin diseases together	**801**	**2408**	**10,2**	**3,0**	**18,3**
Skin diseases other than tumours	**591**	**1134**	**5,2**	**0,9**	**13,2**
Skin infections	376	577	4,8	0,8	13,5
Viral	146	169	2,0	0,2	10,3
Herpes simplex	84	93	1,4	0,1	11,2
Herpes zoster	69	73	2,1	0,2	8,0
Varicella	3	3			
Bacterial	147	207	6,8	2,7	15,5
Folliculitis	31	31	4,4	0,4	14,2
Erysipelas	14	18	10,2	4,9	18,6
Paronychia/Abscess	5	5	14,8	0,2	16,5
Furunculosis	4	4			
Impetigo	4	4			
Sinus pilonidalis	3	3			
Actinomycosis	3	3			
Gas gangrene	2	2			
Catscratch disease	1	1			
Unspecified	105	136	6,5	2,8	15,1
Fungal	91	107	6,3	2,1	14,3
Tinea versicolor	42	42	3,3	1,5	8,5
Dermatomycosis	36	39	8,6	2,1	16,4
Onychomycosis	26	26	12,4	3,5	16,9
Yeast	83	93	2,2	0,1	10,0
Candidiasis	75	84	1,4	0,1	8,9
Pityrosporon folliculitis	7	7	2,5	0,3	6,1
Cryptococcus	1	2			
Parasite	1	1			
Leishmaniasis	1	1			
Inflammatory skin conditions	195	247	4,0	0,9	13,0
Dermatitis	58	62	6,3	1,2	15,4
Seborrhoeic dermatitis	17	17	6,2	0,8	14,5
Sun-induced dermatitis	8	9	2,3	0,3	3,2
Toxic dermatitis	5	5	12,4	7,5	24,7

Asteatotic eczema		4		4			
Nummular eczema		3		3			
Atopic dermatitis		2		2			
Intertriginous eczema		2		2			
Not further determined		20		20	6,7	1,7	16,8
Acne and related diseases	26		35		1,7	0,7	6,1
Acne vulgaris		18		19	1,4	0,8	3,6
Drug-related acne		6		6	1,9	0,5	10,1
Acne conglobata		2		2			
Rosacea		6		6	5,2	0,5	11,6
Perioral dermatitis		2		2			
Alopecia	19		20		0,9	0,4	4,6
Unspecified		8		9	0,8	0,5	2,5
Areata		5		5	4,6	1,3	12,5
Hypotrichosis		2		2			
Cicatricans		2		2			
Mechanica		2		2			
Drug rash	37		38		1,6	0,4	9,8
Pruritus	11		13		11,0	1,8	17,0
Pruritus		9		10	11,2	2,3	17,2
Lichen simplex		3		3			
Psoriasis	4		5		20,9	9,6	21,8
Other	67		74		9,1	1,3	16,2
Vascular skin problems	**138**		**195**		**6,2**	**1,8**	**13,0**
Ulcers	73		90		6,3	1,2	13,1
Gangrene	60		63		5,4	2,2	11,0
Thrombosis	19		19		3,1	0,3	11,1
Varices	14		16		13,6	2,0	20,4
Phlebitis	7		7		13,5	7,2	21,8
Wounds	**37**		**38**		**6,3**	**3,0**	**17,5**
Ùnspecified	36		38		6,3	3,0	17,5
Remainder	**75**		**77**		**5,1**	**0,5**	**12,1**
Edema	6		25		0,7	0,2	10,4
Investigation skin	14		14		11,0	2,2	20,9
Different color skin	20		20		5,6	2,2	10,8
Dyschromia		9		9	4,2	1,3	14,8
Hyperpigmentation		6		6	6,6	2,8	14,9
Hypopigmentation		5		5	5,1	4,6	7,4
Epicutaneous tests	6		8		3,6	0,3	10,8
Hirsutism	3		3				
Unspecified	7		7		8,9	0,7	16,1

Table 1 Distribution of skin diseases between 1994 and 2006 in 591 out of 1768 organ transplant recipients who were transplanted after 1966 and were still alife in 1994, continued.

Skin diseases	Number of patients	Number of diseases	Years after transplantation		
			Median	25 percent	75 percent
Tumours	**456**	**1274**	**14,8**	**7,0**	**22,1**
Benign skin tumours	340	560	9,9	4,2	17,7
Warts (HPV)	152	191	8,6	4,2	15,5
Verruca vulgaris	124	136	11,1	5,0	16,6
Verruca plantaris	18	20	5,6	1,7	10,1
Verruca plana	18	18	7,4	4,9	15,0
Condylomata accuminata	13	15	6,7	2,7	13,9
Verruca filiformis	2	2			
Hyperkeratotic papillomas	60	64	16,9	8,7	23,1
Seborrhoeic keratosis	47	53	8,3	3,0	18,6
Cysts	36	38	9,0	4,0	15,4
Dysplastic nevi	19	20	5,5	2,3	14,7
Hemangioma	5	5	5,3	2,4	23,5
Scars	16	16	7,8	3,0	21,7
Lipoma	9	10	7,5	1,2	16,7
Mollusca (pox virus)	5	5	0,7	0,4	10,3
Other	25	25	9,2	2,9	17,9
Not further characterised	116	133	11,2	5,2	18,2
Premalignant skin lesions	183	275	16,5	9,7	23,3
Actinic keratoses	160	200	15,2	8,8	22,3
Bowen's disease	56	74	19,8	11,1	25,9
Keratoacanthoma	1	1	23,8		
Malignant skin lesions	186	439	17,8	11,5	23,7
Squamous cell carcinoma	130	250	19,5	14,8	24,4
Basal cell carcinoma	101	153	15,9	7,7	23,2
Non melanoma skin cancer unspecified	22	27	15,9	7,0	22,9
Malignant melanoma	4	7	15,5	7,7	19,9
Kaposi's sarcoma	2	2	1,9	1,7	2,1

compared with skin tumours, at a median of 5.2 and 14.8 years after transplantation, respectively (Table 1).

Focusing on the group of skin diseases other than tumours, the most frequently registered diagnoses were skin infections (21.3% of the patients at risk), consisting of viral, bacterial, parasitic, fungal and yeast infections (Table 1). Viral infections (particularly herpes simplex virus and varicella zoster virus) and yeast infections (particularly Candida infections) occurred relatively early, at a median of 2.0 and 2.2 years after transplantation, respectively. The median times to the diagnoses of bacterial and fungal infections were considerably longer, namely 6.8 and 6.3 years after transplantation, respectively. Of the bacterial infections folliculitis was more common during the first years after transplantation, whereas erysipelas was more common after longer post-transplant time periods.

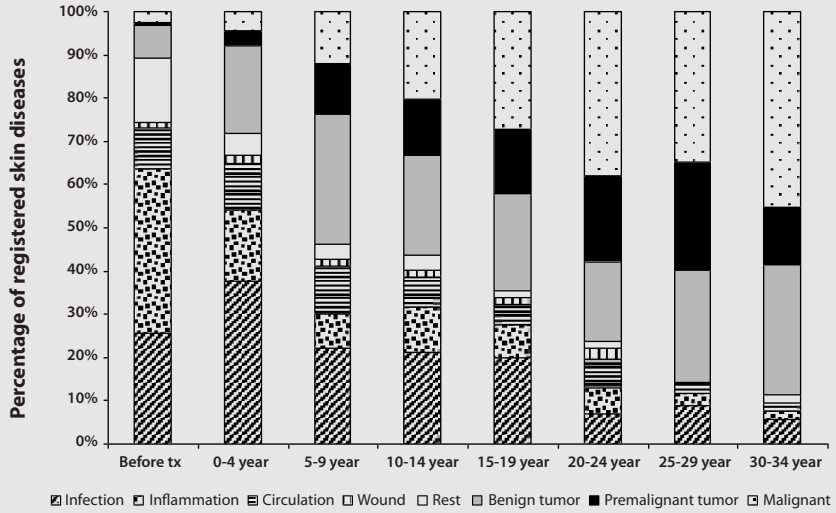
Inflammatory skin conditions were also regularly observed in OTRs (11.0% of the patients at risk), of which dermatitis, acne and drug-related rashes were the most frequently registered skin diseases. As a consequence of the immunosuppressive therapy, acne vulgaris and drug rashes developed shortly after transplantation, whereas rosacea was seen later after approximately 5 years. Vascular skin problems, mainly resulting in ulcers and gangrene, had been registered in 138 patients (7.8% of the patients at risk) with a total number of 195 diagnoses and a median time after transplantation of 6.2 years (Table 1).

Focusing on the group of registered skin tumours, 44.0% of the tumours were benign (19.2% of the patients at risk), 21.6% premalignant (10.4% of the patients at risk), and 34.4% were malignant (10.5% of the patients at risk). The median time from transplantation to the registration of the benign tumours was 9.9 years. The median time from transplantation to the premalignant and malignant tumours was much longer, 16.5 and 17.8 years, respectively (Table 1). The most frequently diagnosed benign skin lesions were HPV-related warts (verrucae) and the most frequently diagnosed premalignant lesions were actinic keratoses (Table 1). HPV-related warts occurred earlier, with a median time after transplantation of 8.6 years, compared with actinic keratoses (15.2 years). In turn, actinic keratoses preceded the development of malignant tumours (Table 1). The number of registered keratoacanthomas was remarkably low, which probably reflects the simultaneous occurrence of SCCs in patients with keratoacanthomas and the fact that only the clinically most relevant diagnosis was registered. More than half of the malignant skin lesions were SCCs and roughly one third were BCCs. Furthermore, BCCs appeared to occur about 3.5 years earlier compared with SCCs (Table 1).

Registered skin diseases and histological diagnoses in relation to time after transplantation

Figure 1 shows the relative contribution of registered skin diseases according to the time period after transplantation. In 93 of 1768 patients (5.3%) altogether 157 skin diseases had been registered before transplantation. With 784 registered skin diseases, the period between 0 to 4 years after transplantation showed the highest absolute number. The absolute numbers of registered skin diseases decreased later after transplantation as a consequence of decreasing numbers of patients at risk during the later time categories (Figure 1). The percentage of patients with registered skin diseases ranged between 22 and 35%, during the different time periods and the mean number of skin diseases per patient ranged between 1.8 and 3.1 during these time periods.

Figure 1 Relative distribution of registered skin diseases in patients with one or more skin diseases in relation to different time periods after transplantation.



Years after transplantation	before	0-4 year	5-9 year	10-14 year	15-19 year	20-24 year	25-29 year	30-34 year
Number of patients at risk (max-min)	1768	1768-916	916-678	678-493	493-359	359-191	191-91	91-22
Number of patients with registered skin diseases	93	429	201	172	136	103	54	22
Number of skin diseases	137	784	407	371	359	245	169	53
Mean number of skin diseases per patient	1.7	1.8	2.0	2.2	2.6	2.4	3.1	2.4

Within the first 4 years after transplantation, infections and inflammatory conditions accounted for > 50% of all registered skin diseases and this contribution substantially decreased with time to < 10% at 30 years after transplantation (Figure 1). The relative contribution of benign tumours was stable over time and ranged between 20 and 30%. This is in contrast to the premalignant and malignant skin lesions, which contributions rose from 3 to 20% and from 4 to 45%, respectively, with increasing time after transplantation (Figure 1). When the same analyses were performed after stratification for age (patients younger or older than 50 years) and sex similar trends were observed (data not shown).

Discussion

This study gives a systematic overview of the high burden of skin diseases in OTR. Each year > 10% of the patients were diagnosed with a skin disease and during a 13-year period, 48% of the patients had developed one or more skin diseases. Many patients developed multiple or recurrent skin diseases.

The spectrum of skin diseases changed considerably with increasing time after transplantation. The first post-transplant years were dominated by skin infections such as herpes simplex, herpes zoster and Candida infection, and inflammatory skin diseases such as acne and skin rashes. In the later post-transplant years premalignant and malignant skin tumours started to prevail at the expense of infections and inflammatory diseases. The median time period after transplantation to the diagnoses of common warts was 11.1 years, to actinic keratoses 15.2 years, to BCC 15.9 years and to SCC 19.5 years. It is well known that due to the use of immunosuppressive drugs OTRs are frequently infected by HPV [18;19;25;26]. Interestingly, the prevailing benign (warts), premalignant (actinic keratoses) and malignant skin tumours (SCC) are all known or thought to be associated with HPV infection [18;19;25;26].

This study confirmed earlier publications that skin infections occur early after transplantation [9;10;21], and that skin cancers increase exponentially with increasing time after transplantation [2;3;6;12;22]. Little is known about vascular skin problems after organ transplantation. Our study showed that 138 (7.8%) of 1768 OTRs had some type of skin condition related to vascular problems. Both arterial and venous vascular complications have been described in renal transplant recipients [23;24] and also simultaneous pancreas and kidney transplant recipients are at increased risk for vascular problems as a consequence of many years of poorly regulated levels of glucose. To estimate the cumulative incidence of vascular skin problems in organ transplant recipients, however, additional cohort studies will be necessary.

The LUMC diagnostic registration system has the disadvantage that from 2005 onwards only one diagnosis per patient per year was allowed to be registered and that the registration of skin diseases before 2005 was not always inclusive. The numbers of skin diseases presented in this study, therefore, are an underestimation of the real number and the type of diagnoses may be biased towards more severe diagnoses, such as malignant skin tumours. Although most patients who are transplanted at our hospital are regularly seen at the Department of Nephrology and those with cutaneous problems are also seen at the Department of Dermatology, we cannot exclude that some patients were also seen in other dermatology clinics, which may have led to an additional underestimation of the total number of registered skin disease. Finally, not every patient will consult a dermatologist for every skin disease, in particular when these diseases have few medical consequences, which forms an additional source of underreporting.

In conclusion, the frequency of skin diseases in OTR is high, especially if one considers that the number of infections in this study probably represents only the tip of the iceberg of the real incidence of skin infections in OTR. Therefore, OTRs should be regularly checked by trained dermatologists and given a careful skin examination so that skin diseases can be treated at an early stage.

Acknowledgement

The authors thank Jan Molenaar and Koos Mistrate Haarhuis for providing important clinical data. We are also grateful to Paul Douw van der Krap for his support in laying out the figure. M.C.W. Feltkamp is supported by The Dutch Organisation for Health Research and Development (ZonMW Clinical Fellowship 907-00-150).

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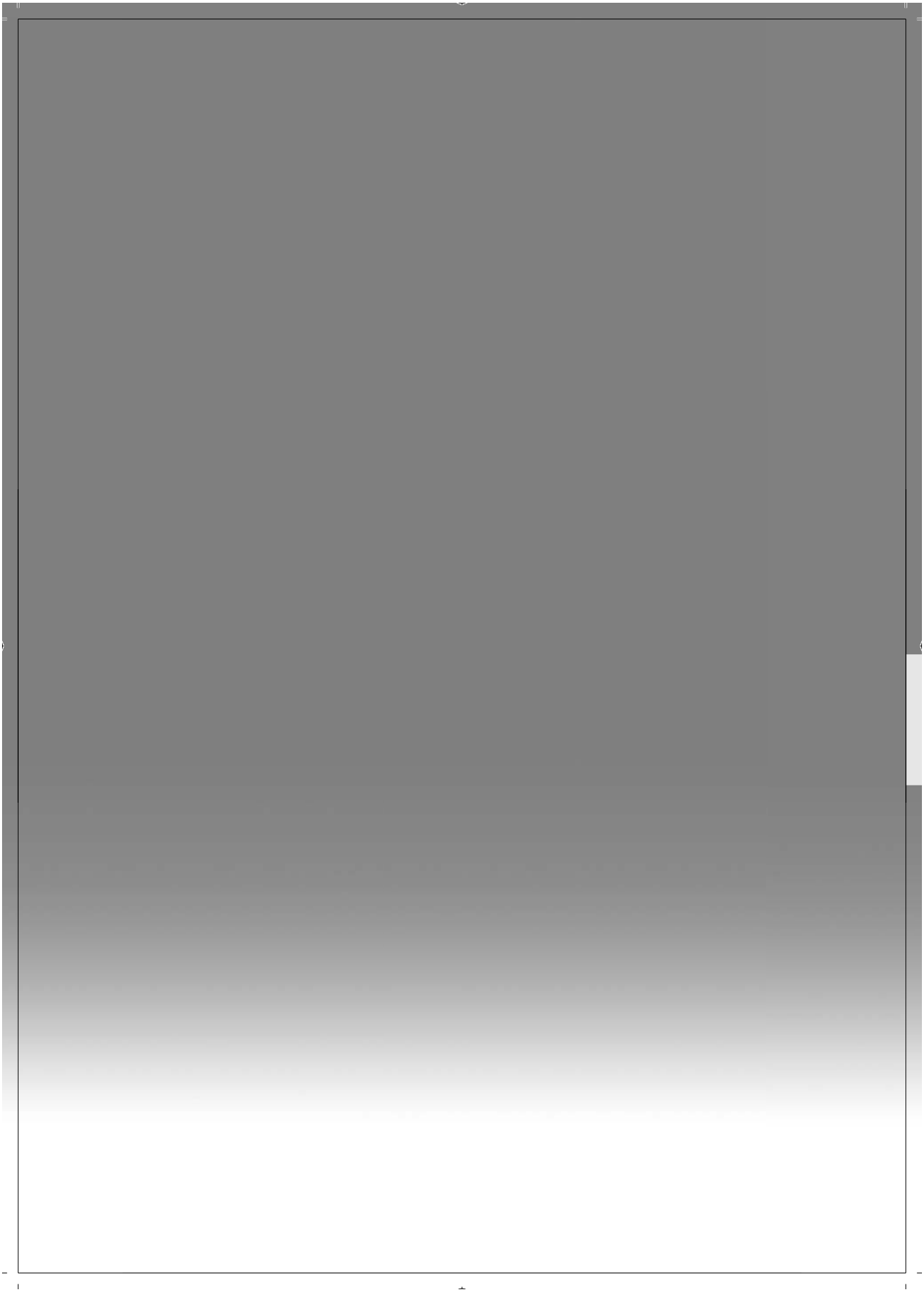

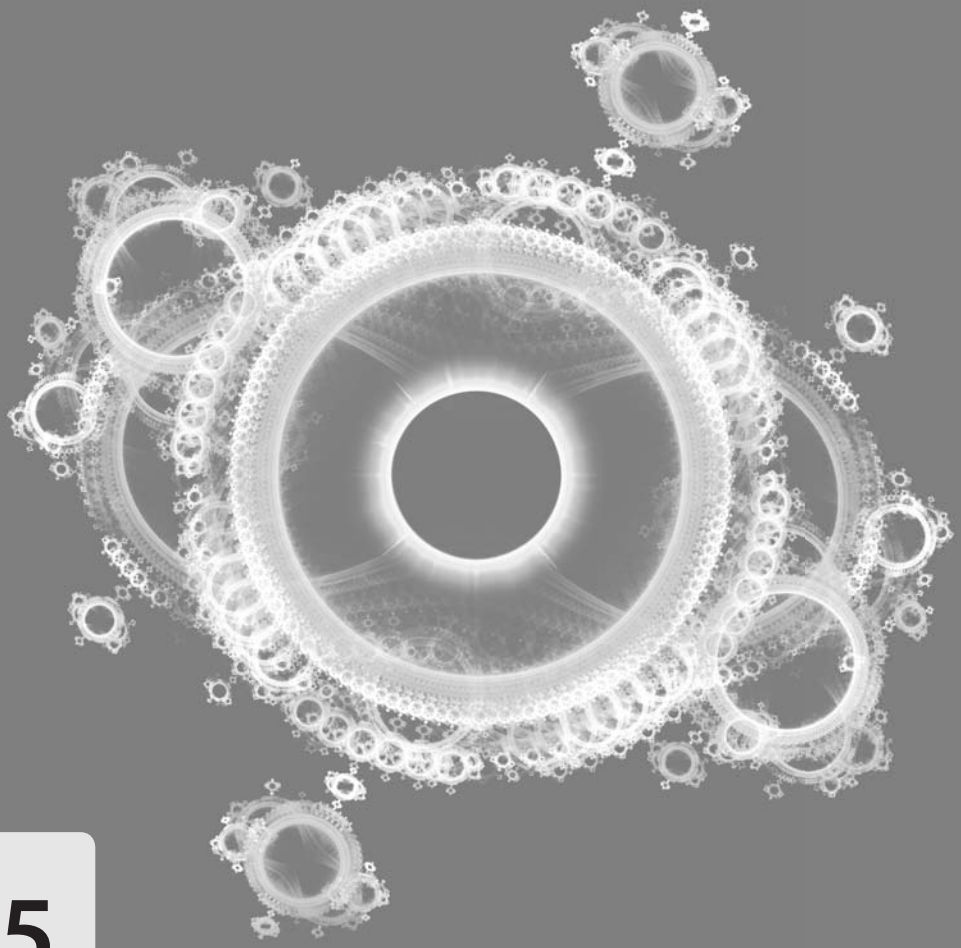

5

Increased risk of squamous-cell carcinoma in simultaneous pancreas kidney transplant recipients compared with kidney transplant recipients

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Journal of Investigative Dermatology 2010;162(2):390-6

Abstract

The purpose of this study was to ascertain the risk of non-melanocytic skin cancer (NMSC) in simultaneous pancreas kidney transplant recipients (SPKTRs) compared to kidney transplant recipients (KTRs) in relation to other potential risk factors of skin cancer. In a cohort study, 208 SPKTRs were compared with 1,111 KTRs who were transplanted during the same time period. The effects of age, sex, country of origin, time period after transplantation, HLA matching, immunosuppressive regimen and rejection treatments on the risk of NMSC were investigated in multivariable Cox's proportional hazard models. In SPKTRs the incidence of NMSC increased from 19 to 36%, respectively 10 and 15 years after transplantation which was significantly higher compared with that in KTRs (6 and 10%, respectively). After adjustment for age and sex, SPKTRs had a 6.2 (3.0-12.8) increased risk of squamous-cell carcinoma (SCC) compared to KTRs. An additional adjustment for maintenance immunosuppression decreased the hazard ratio to 3.1 (1.3-7.2) which indicates partial confounding by the immunosuppressive regimen. Adjustment for induction and rejection therapy or HLA mismatching did not change the hazard ratio significantly. SPKTRs have an increased risk of SCC compared with KTRs, despite partial confounding by the immunosuppressive regimen.

Introduction

Organ-transplant recipients are at increased risk for post-transplant neoplasms (Hardie *et al*, 1980; Hartevelt *et al*, 1990). Non-melanocytic skin cancers (NMSCs), especially squamous-cell carcinomas (SCC), are the most common malignancies and can cause substantial morbidity and even mortality (Hartevelt *et al*, 1990; Bouwes Bavinck *et al*, 1996; Naldi *et al*, 2000; Jensen *et al*, 2000; Euvrard *et al*, 2003; Otley *et al*, 2005b; Moloney *et al*, 2006).

Increasing age, male sex, and fair complexion are the most important host-related risk factors for skin cancer, and exposure to sunlight, smoking and infection with human papillomaviruses are the most important environmental risk factors (De Hertog *et al*, 2001; Kasiske *et al*, 2004; Bouwes Bavinck and Feltkamp, 2004; Bouwes Bavinck *et al*, 2008). Among organ-transplant recipients, immunosuppressive therapy forms an additional important risk factor (Hartevelt *et al*, 1990; Bouwes Bavinck *et al*, 2007). Both the duration and type of immunosuppression may play a role. Azathioprine (Aza) has been reported to induce selective UVA photosensitivity, which may result in a cascade of reactions in the skin, ranging from the induction of oxidative stress and mutagenic DNA lesions to the development of skin cancer (O'Donovan *et al*, 2005; Ulrich and Stockfleth, 2007; Cooke *et al*, 2007; Montaner *et al*, 2007). Cyclosporine A (CsA) can decrease DNA repair and impair UV-induced apoptosis, which also increases the risk of skin cancer (Yarosh *et al*, 2005). Poor HLA matching has been reported to be associated with an increased risk of NMSC (Bouwes Bavinck *et al*, 1991).

Among kidney-transplant recipients (KTRs) living in a temperate climate, the prevalence of NMSC at 10 years after transplantation varied between 10 and 27% and at 20 years between 40 and 60% (Hartevelt *et al*, 1990; Bordea *et al*, 2004; Moloney *et al*, 2006). In Australia, the incidence is even higher (Hardie *et al*, 1980; Bouwes Bavinck *et al*, 1996; Ramsay *et al*, 2002). Heart-transplant recipients seem to have a higher incidence of NMSC compared with KTRs, although this may be a consequence of older age at transplantation in this group (Mihalov *et al*, 1996; Naldi *et al*, 2000; Fortina *et al*, 2000). Less research has been conducted in patients receiving a liver transplant. After a follow-up period of 10 years, an incidence between 13 and 26% has been found in Dutch and Spanish liver-transplant recipients, respectively (Haagsma *et al*, 2001; Herrero *et al*, 2005). There are no studies that followed up lung-transplant recipients or simultaneous pancreas kidney transplant recipients (SPKTRs) for a longer period.

Since 1986, simultaneous pancreas kidney transplantations (SPKTs) are being performed in the Netherlands. At present, more than 200 patients received an SPKT at

the Leiden University Medical Center (LUMC). The main objective of this study was to calculate the cumulative incidence of skin cancer in SPKTRs compared with the incidence in KTRs who were transplanted in the same center during the same time period.

We hypothesized that the risk of skin cancer in SPKTRs would be higher compared with that in KTRs, because SPKTRs are exposed to a more potent immunosuppressive regimen and are not HLA-matched in contrast to KTRs.

Results

Baseline characteristics of the KTR and SPKTR

The baseline characteristics of the KTRs and SPKTRs are depicted in Table 1. The majority of the patients originated from the Netherlands. In the KTR group, there were significantly more patients originating from Mediterranean countries or from countries that are associated with a darker skin type (Table 1). Sex distribution did not differ significantly between the two groups, but the SPKTRs were on an average 7.4 years younger at first transplantation than were the KTRs ($P < 0.001$). The median follow-up time of the SPKTRs was shorter ($P = 0.014$), because, during the first few years, the number of SPKTs was still limited (Table 1). After adjustment for age, sex and immunosuppressive therapy, overall survival was significantly shorter for SKPTRs compared with KTRs, with an adjusted hazard ratio of 2.1 (1.5-3.1).

Cumulative incidence of skin cancer in the SPKTR compared with that in the KTR

The baseline characteristics of the KTRs and SPKTRs in relation to the development of SCC and basal-cell carcinoma (BCC) as first events are depicted in Table 2, and potential risk factors for NMSC, SCC and BCC are presented for KTRs and SPKTRs, separately, in Supplementary Figure S1 and Supplementary Tables S1a and S1b. Two of the KTRs had developed an SCC and a BCC and four only a BCC before transplantation. These skin cancers were not considered in the analyses. None of the SPKTRs had developed an SCC or a BCC before transplantation. The time period after transplantation was significantly associated with the occurrence of SCC and BCC ($P < 0.001$), but sex was not associated with skin cancer (Table 2). In the Cox's proportional hazard model, increasing age at transplantation was a risk factor for both types of skin cancer (Supplementary Tables S1a and S1b).

During the follow-up period until June 2007, a total of 109 skin cancers (73 SCCs and 36 BCCs) were diagnosed in 26 (12.5%) out of 208 SPKTRs (Table 2). During the

Table 1 Baseline characteristics of 1111 kidney transplant recipients and 208 simultaneous pancreas kidney transplant recipients.

	KTR*	SPKTR*	P value
No of patients	**1111**	**208**	
Country of origin			
Netherlands	973 (87.6)	203 (97.6)	
Mediterranean	58 (5.2)	3 (1.4)	P < 0.001
Suriname, Africa, Asia	80 (7.2)	2 (1.0)	
Male: N (%)	690 (62.1)	126 (60.6)	P = 0.677
Age at transplant (years)			
Median	48.6	40.5	P < 0.001
25% - 75%	37.8 – 58.5	34.8 – 46.0	
Follow-up (years)			
Median	6.9	6.4	P = 0.014
25% - 75%	3.6 – 12.1	3.5 – 10.1	
HLA mismatches			
0	178 (16.1)	1 (0.5)	
1-3	774 (70.2)	52 (25.0)	P < 0.001
4-6	151 (13.7)	155 (74.5)	
Unknown	8	0	
Death: N (%)	363 (33.0)	63 (30.4)	P = 0.475**
Unknown	10	1	

*KTR = kidney transplant recipient, SPKTR = simultaneous pancreas kidney transplant recipient.
** After adjustment for age, sex and immunosuppressive therapy overall survival was significantly shorter for SKPTR compared to KTR with an adjusted hazard ratio of 2.1 (1.5;3.1).

same follow-up period, 68 (6.1%) out of 1,111 KTRs developed altogether 223 skin cancers (102 SCCs and 121 BCCs). The overall SCC:BCC ratio in the KTR was 0.79. This ratio gradually increased with increasing time after transplantation with ratios of 0.67, 0.55, 0.71, and 1.0 during the first 2, 2-7, 8-12, and 13-17 years after transplantation, respectively. The overall SCC:BCC ratio in the SPKTR was 1.1. The ratios were 0, 1.1, and 1.4 during the periods between 2-7, 8-12, and 13-17 years after transplantation, respectively.

The cumulative incidences of NMSC, SCC and BCC in SPKTRs are compared with those in KTRs in Figure 1 and Supplementary Figure S2.

Table 2 Baseline characteristics according to the presence of non-melanocytic skin cancer and risk factors of skin cancer in kidney transplant recipients and simultaneous pancreas kidney transplant recipients.

	KTR*			SPKTR*			
Type of skin cancer	**No skin cancer (censored)**	**SCC* as first event**	**BCC* as first event**	**P-value**	**No skin cancer (censored)**	**SCC* as first event**	**BCC* as first event**	**P-value**
No. of patients: N (%)	1043 (94.0)	28 (2.5)	40 (3.6)		182 (87.5)	14 (6.7)	12 (5.8)	
No. of patients with SCC	0	28	10		0	14	4	
No. of patients with BCC	0	8	40		0	5	12	
SCC per pat with NMSC								
Mean (Min-Max)	0	2.5 (1-7)	0.8 (0-7)		0	4.9 (1-14)	0.3 (0-1)	
BCC per pat with NMSC								
Mean (Min-Max)	0	0.5 (0-5)	2.7 (1-20)		0	0.4 (0-2)	2.5 (1-12)	
Country of origin								
Netherlands	907 (87.0)	28 (100)	38 (95.0)		177 (97.3)	14 (100)	12 (100)	
Mediterranean	57 (5.5)	0	1 (2.5)	Pscc = 0.124	3 (1.6)	0	0	Pscc = 0.821
Africa, Asia	79 (7.5)	0	1 (2.5)	Pbcc = 0.323	2 (1.1)	0	0	Pbcc = 0.844
Male: N (%)	647 (62.0)	20 (71.4)	23 (57.5)	Pscc = 0.311 Pbcc = 0.562	109 (59.9)	9 (64.3)	8 (66.7)	Pscc = 0.746 Pbcc = 0.642
Age at transplant (years)								
Median	48.4	51.9	49.6	Pscc = 0.147	40.6	42.5	37.4	Pscc = 0.933
25% - 75%	37.7 – 58.1	40.9 – 61.7	44.8 – 60.2	Pbcc = 0.226	34.8 – 46.0	33.6 – 47.8	33.3 – 48.1	Pbcc = 0.566
Follow-up (years)								
Median	6.6	13.0	11.6	Pscc < 0.001	5.6	14.0	10.3	Pscc < 0.001
25% - 75%	3.5 – 11.6	9.5 – 19.3	7.8 – 16.8	Pbcc < 0.001	3.1 – 8.7	12.4 – 15.8	8.5 – 14.9	Pbcc < 0.001
HLA mismatches								
0	165 (15.9)	4 (14.3)	9 (22.5)		0	1 (7.1)	0	
1-3	728 (70.3)	23 (82.1)	23 (57.5)	Pscc = 0.263	46 (25.3)	4 (28.6)	2 (16.7)	Pscc = 0.391#
4-6	142 (13.7)	1 (3.6)	8 (20.0)	Pbcc = 0.221	136 (74.7)	9 (64.3)	10 (83.3)	Pbcc = 0.503
Unknown	8	0	0		0	0	0	

ATG or OKT3 as induction or rejection treatment								
No	760 (72.9)	21 (75.0)	30 (75.0)	Pscc = 0.802	44 (24.2)	2 (14.3)	1 (8.3)	Pscc = 0.400
Yes	283 (27.1)	7 (25.0)	10 (25.0)	Pbcc = 0.766	138 (75.8)	12 (85.7)	11 (91.7)	Pbcc = 0.208
Type of maintenance immunosuppression**								
P + Aza	70 (6.7)	9 (32.1)	5 (12.5)		0	0	0	
P + CsA	429 (41.3)	15 (53.6)	23 (57.5)		0	0	0	
P + MMF	95 (9.2)	2 (7.1)	2 (5.0)		0	0	0	
P + Tac	38 (3.7)	0	0	Pscc < 0.001	0	0	0	Pscc < 0.001
P + Aza + CsA	5 (0.5)	0	1 (2.5)	Pbcc = 0.064	64 (35.2)	14 (100)	5 (41.7)	Pbcc = 0.218
P + MMF + CsA	275 (26.5)	2 (7.1)	6 (15.0)		81 (44.5)	0	7 (58.3)	
P + MMF + Tac	126 (12.1)	0	3 (7.5)		37 (20.3)	0	0	
Unknown	5	0	0		0	0	0	
Level of immunosuppression***								
Low	419 (40.2)	20 (71.4)	25 (62.5)		0	0	0	
Moderate	561 (53.8)	7 (25.0)	11 (27.5)	Pscc = 0.004	44 (24.2)	2 (14.3)	1 (8.3)	Pscc = 0.627
High	63 (6.0)	1 (3.6)	4 (10.0)	Pbcc = 0.005	111 (61.0)	9 (64.3)	8 (66.7)	Pbcc = 0.362
Very high	0	0	0		27 (14.8)	3 (21.4)	3 (25.0)	

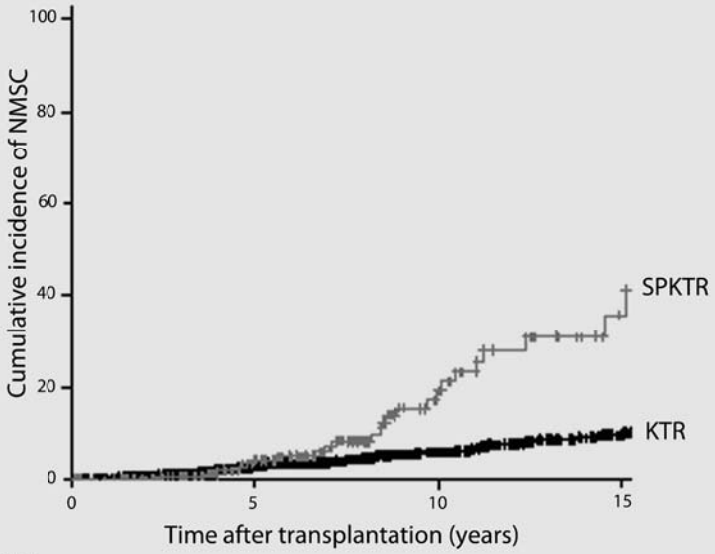
*KTR = kidney transplant recipient, SPKTR = simultaneous pancreas kidney transplant recipient, SCC = squamous-cell carcinoma, BCC = basal-cell carcinoma.

#For calculation of this p-value the numbers for 0 – 3 mismatches were combined.

**P = prednisolone; Aza = azathioprine; CsA = cyclosporine A; MMF =mycofenolatemofetil; Tac =tacrolimus.

*** The level of immunosuppression is defined in the methods.

Figure 1 Cumulative incidence of skin cancer in 208 simultaneous pancreas kidney transplant recipients (SPKTR) compared to 1111 kidney transplant recipients (KTR). The numbers of SPKTR and KTR at risk in relation to the years after transplantation are indicated in the Table.



Number af risk				
SPKTR	208	121	42	12
KTR	1105	691	356	147

Possible risk factors for skin cancer

To identify the possible factors that could explain the increased risk of skin cancer among SPKTRs compared with KTRs, we analyzed the influence of age, sex, country of origin, HLA matching, maintenance immunosuppressive regimen, induction and rejection treatments, and level of immunosuppression on the risk of skin cancer within the SPKTRs and KTRs (Supplementary Figure S1 and Supplementary Tables S1a and S1b).

HLA matching and skin cancer

No HLA matching is carried out in SPKTRs. Therefore, the number of mismatches was much higher among the SPKTRs than in KTRs (Table 1). HLA mismatching, however,

was not significantly associated with SCC or BCC in either the KTRs or the SPKTRs (Table 2, Supplementary Figure S1f and Supplementary Tables S1a and S1b).

Immunosuppressive regimens and skin cancer

The immunosuppressive regimens differed strongly between SPKTRs and KTRs, and changed considerably during the years (Table 3). SPKTRs always received triple therapy, whereas this regimen was introduced much later in KTRs (Table 3).

In both KTRs and SPKTRs, immunosuppressive regimens were associated with the development of SCC but not of BCC (Table 2, Supplementary Figure S1e and Supplementary Tables S1a and S1b). For the main analyses, the immunosuppressive regimens were categorized into three basic treatment groups: Aza in any combination, mycophenolatemofetil (MMF) in any combination, or CsA or tacrolimus (Tac) without Aza or MMF.

In the KTR group, immunosuppression with MMF compared with that with Aza was associated with a significantly decreased risk of SCC (Supplementary Figure S1e, SCC). The hazard ratio adjusted for age and sex was 0.15 (0.04-0.59) (Supplementary Table S1a). Additional adjustments for the simultaneous use of CsA; for triple versus duo therapy or for the number of HLA mismatches did not change this hazard ratio significantly. In the KTR group, immunosuppression with CsA was also associated with a significantly decreased risk of SCC compared with Aza (Supplementary Figure S1e, SCC). The hazard ratio adjusted for age and sex was 0.35 (0.15-0.84) (Supplementary Table S1a).

In the SPKTR group, immunosuppression with MMF compared with that with Aza was also associated with a decreased risk of SCC (Supplementary Figure S1e, SCC). The hazard ratio could not be calculated, however, because all SCC cases were immunosuppressed with Aza in any combination and none with MMF in any combination (Table 2 and Supplementary Table S1b). SPKTRs who had maintenance therapy with MMF in any combination seemed to have an increased risk of BCC compared with patients who were using maintenance therapy with Aza in any combination, although statistical significance was not reached, and this increased risk was not observed in KTRs (Supplementary Figure S1e, BCC). As almost all SPKTRs were immunosuppressed with CsA, either in combination with prednisolone and Aza or with prednisolone and MMF (Table 2), the risk of SCC associated with the use of CsA could not be calculated in the SPKTR group.

Table 3 Immunosuppressive therapy of 1111 kidney transplant recipients and 208 simultaneous pancreas kidney transplant recipients according to time periods of transplantation, corresponding with major changes in immunosuppressive regimen.

	KTR*				SPKTR*			
Transplantation period	**1986 - 1995**	**1996 – 2001**	**2002 - 2005**	**P-value**	**1986 - 1995**	**1996 – 2001**	**2002 - 2005**	**P-value**
No of patients*	530	289	292		72	77	59	
SCC total (1st event)	34 (25)	3 (2)	1 (1)		16 (13)	2 (1)	0 (0)	
BCC total (1st event)	33 (28)	10 (8)	5 (4)		10 (5)	7 (7)	0 (0)	
ATG or OKT3 as induction or rejection treatment								
No	327 (61.7)	223 (77.2)	261 (89.4)	P < 0.001	11 (15.3)	22 (28.6)	14 (23.7)	P = 0.148
Yes	203 (38.3)	66 (22.8)	31 (10.6)		61 (84.7)	55 (71.4)	45 (76.3)	
Type of maintenance immunosuppression**	N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
P + Aza	83 (15.7)	1 (0.3)	0		0	0	0	
P + CsA	374 (70.8)	86 (29.9)	7 (2.4)		0	0	0	
P + MMF	37 (7.0)	41 (14.2)	21 (7.2)		0	0	0	
P + Tac	1 (0.2)	27 (9.4)	10 (3.4)	P < 0.001	0	0	0	P < 0.001
P + Aza + CsA	6 (1.1)	0	0		72 (100)	8 (10.4)	3 (5.1)	
P + MMF + CsA	26 (4.9)	99 (34.3)	158 (54.5)		0	69 (89.6)	19 (32.2)	
P + MMF + Tac	1 (0.2)	34 (11.8)	94 (32.4)		0	0	37 (62.7)	
Unknown	2	1	2		0	0	0	

Level of immunosuppression***							
Low	307 (57.9)	124 (42.9)	33 (11.3)		0	0	0
Moderate	211 (39.8)	132 (45.7)	236 (80.8)	P < 0.001	11 (15.3)	22 (28.6)	14 (23.7)
High	12 (2.3)	33 (11.4)	23 (6.9)		45 (62.5)	47 (61.0)	36 (61.0)
Very high	0	0	0		16 (22.2)	8 (10.4)	9 (15.3)
							P = 0.179

*KTR = kidney transplant recipient; SPKTR = simultaneous pancreas kidney transplant recipient; SCC = squamous-cell carcinoma, BCC = basal-cell carcinoma..
**P = prednisolone; Aza = azathioprine; CsA = cyclosporine A; MMF =mycofenolatemofetil; Tac =tacrolimus.
*** The level of immunosuppression is defined in the methods.

Induction and rejection treatments and level of immunosuppression in relation to skin cancer

Among SPKTRs, induction or rejection treatments with antithymocyte globulin (ATG) or muromonab (OKT3) were not associated with an increased risk of NMSC, SCC or BCC (Supplementary Figure S1h-j). The hazard ratios adjusted for age, sex and immunosuppressive therapy for induction and rejection treatments to develop NMSC were 0.91 (0.38-2.2) and 1.5 (0.42-5.4), respectively. For SCC, the adjusted hazard ratios were 0.92 (0.29-3.0) and 1.3 (0.15-10.1), respectively, and for BCC they were 0.68 (0.18-2.6) and 2.4 (0.49-12.1), respectively.

Owing of insufficient numbers of induction treatments among KTRs in this subgroup, we could only calculate the hazard ratios for rejection treatments. The adjusted hazard ratios were 0.75 (0.42-1.4), 0.63 (0.25-1.6), and 0.83 (0.38-1.8) for NMSC, SCC and BCC, respectively.

As the biological effects of ATG and/or OKT3 are supposed to be similar before and after the transplantation, induction and rejection treatments with ATG and/or OKT3 were combined. Treatment with ATG and/or OKT3 at any time was not significantly associated with the development of NMSC, SCC or BCC in this study (Supplementary Figure S1j and Supplementary Tables S1a and S1b).

Triple therapy and treatment with ATG and/or OKT3 are the most important factors determining the level of immunosuppression. By combining these treatment modalities, we estimated a "general" level of immunosuppression. Using this estimation, the level of immunosuppression was not consistently associated with NMSC, SCC or BCC (Supplementary Figure S1k and Supplementary Tables S1a and S1b). In the SPKTR, we also calculated the median daily

doses of prednisone, Aza, MMF, CsA and Tac, none of which were associated with skin cancer (data not shown).

SPKTRs have an increased risk of SCC compared with KTRs, which can be partly explained by confounding by an immunosuppressive regimen

Non-stratified Kaplan-Meier analyses and analyses stratified for potentially confounding factors are shown in Supplementary Figure S2 and non-adjusted and adjusted hazard ratios of developing NMSC, SCC or BCC in SPKTRs compared with those in KTRs are presented in Table 4.

Table 4 Risk of skin cancer in simultaneous pancreas kidney transplant recipients compared to kidney transplant recipients with adjustment for potentially confounding factors using Cox proportional hazard analyses.

Adjustments	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
No adjustment	*3.0 (1.9;4.8)*	*4.2 (2.2;8.1)*	*2.5 (1.3;4.9)*
Age	4.0 (2.4;6.5)	6.3 (3.1;13.0)	3.1 (1.5;6.1)
Sex	3.0 (1.9;4.8)	4.1 (2.1;8.0)	2.5 (1.3;4.9)
Age and sex	*4.0 (2.4;6.5)*	*6.2 (3.0;12.8)*	*3.1 (1.5;6.2)*
Age, sex and country of origin*	3.8 (2.3;6.2)	5.7 (2.8;11.8)	3.0 (1.5;6.0)
Age, sex and HLA mismatching**	3.3 (1.7;6.3)	8.3 (3.4;20.2)	1.7 (0.72;4.0)
Age, sex and maintenance immunosuppression***	3.0 (1.7;5.5)	3.1 (1.3;7.2)	3.1 (1.4;6.9)
Age, sex and ATG or OKT3 as induction or rejection treatment	3.9 (2.3;6.7)	6.3 (2.9;13.9)	2.9 (1.4;6.2)
Age, sex and level of immunosuppression****	2.4 (1.0;5.9)	6.5 (1.7;25.3)	1.3 (0.43;4.0)
Age, sex, HLA mismatching and maintenance immunosuppression	2.5 (1.2;5.1)	3.8 (1.4;10.2)	1.8 (0.68;4.5)

*Netherlands and neighbor countries; Mediterranean countries; or Suriname, Africa or Asia.
**No; 1-3; or 4-6 HLA A, B and DR mismatches.
***Aza in any combination; MMF in any combination; or CsA or Tac without Aza or MMF,
****Low, moderate, high or very high immunosuppression as explained in the methods.

The Kaplan-Meier analyses show an increased risk of SCC in SPKTRs compared with that in KTRs in almost all strata (Supplementary Figure S2). Supplementary Figure S2d shows that SPKTRs were much younger at transplantation than were KTRs. Adjustment for age, therefore, increased the hazard ratio for the association between transplanted organ and SCC (Table 4). Supplementary Figure S2f shows that risk of SCC was much lower in the group of patients who were immunosuppressed with MMF in any combination. Adjustment for maintenance immunosuppression decreased the hazard ratio for the association between transplanted organ and SCC, which was adjusted for age and sex from 6.2 (3.0-12.8) to 3.1 (1.3-7.2), which suggests a partial confounding by maintenance immunosuppression (Table 4). Adjustment for other potentially confounding factors did not reduce the hazard ratios for SCC notably (Table 4).

The risk of BCC in SPKTR compared with that in KTR was reduced after adjustment for HLA mismatching and for the level of immunosuppression, and, when all relevant potentially confounding factors were introduced into the Cox's proportional hazard model, the increased risk of BCC largely disappeared (Table 4).

Discussion

This study showed, after adjustment for age and sex, a 6.2-fold (95% CI: 3.0-12.8) increased risk of SCC in SPKTRs than in KTRs who were transplanted in the same center during the same time period. After an additional adjustment for maintenance immunosuppression, this risk decreased to 3.1 (1.3-7.2). The risk of BCC was not statistically significantly increased in SPKTRs after adjustment for potentially confounding factors.

Maintenance immunosuppressive therapy with MMF in any combination had led to a significantly decreased risk of SCC compared with maintenance immunosuppressive therapy with Aza. SPKTRs were more often immunosuppressed with Aza than were KTRs. Adjustment for this factor, indeed, reduced the risk of SCC in SPKTRs compared with that in KTRs, suggesting that the increased risk of SCC in SPKTR can be partly explained by confounding by the type of maintenance immunosuppressive therapy. There remained, however, a statistically significant three-fold increased risk of SCC in SPKTR, for which we looked for other potential explanations.

Apart from an obligate history of diabetes in the SPKTRs and differences in maintenance immunosuppression, other differences discerning SPKTRs from KTRs are more frequent induction and rejection therapies, and the absence of HLA matching

in SPKTRs. Moreover, these factors could potentially explain the increased risk of SCC in SPKTRs compared with that in KTRs.

The incidence of NMSC in patients with type 1 diabetes has not been systematically studied (Zendehdel *et al*, 2003; Swerdlow *et al*, 2005). Only Zendehdel *et al* (2003) showed a modest, but statistically nonsignificant increase of NMSC, with a standardized incidence ratio of 1.9 (0.6-4.3) in patients who had type 1 diabetes mellitus for more than 15 years (Zendehdel *et al*, 2003). In organ-transplant recipients, diabetes was associated with a decreased risk of NMSC (Kasiske *et al*, 2004; Otley *et al*, 2005a). It is therefore not likely that type 1 diabetes may explain the increased risk of SCC among SPKTRs.

Induction treatments, impending graft rejection, and the subsequent rejection therapies were not associated with SCC or BCC in this study, although the follow-up periods may still have been too short to detect such an effect. Adjustment for induction and rejection treatments did not change the increased risk of SCC in SPKTRs, excluding also these factors as major causes for the increased risk of SCC in SPKTRs.
Although HLA matching has been reported to be associated with skin cancer in an earlier study (Bouwes Bavinck *et al*, 1991), we were not able to confirm this association in this study. Adjustment for HLA matching did not influence the risk of SCC among SPKTRs; hence, poor HLA matching could not explain the increased risk of SCC in SPKTRs. The risk of BCC in SPKTRs, compared with that in KTRs, however, decreased after adjustment for HLA matching, suggesting that poor HLA matching could partly explain the increased risk of BCC in SPKTRs.

Differences in the number of induction and graft rejection treatments, as well as HLA matching, did not provide a good explanation for the increased risk of SCC in SPKTRs compared with KTRs. However, other differences between the two groups might be responsible for this outcome. Compared with KTRs, in SPKTRs, a second transplanted organ is present. Induction of tolerance is an important goal of clinical organ transplantation (Kean *et al*, 2006; Kawai *et al*, 2008), and may also have undesirable side effects, such as an increased risk of skin cancer. We speculate that transplanted pancreas may induce tolerance against an additional set of allo-peptides in the HLA antigens of the donor. Although we are not aware of any published examples of this mechanism in humans who have received a double set of other organs (for example, heart and lung), a reduced rejection rate of the transplanted heart has been described in rats who received a heart in combination with a lung or spleen (Westra *et al*, 1991). An increased cross-reactive tolerance against SCC-associated antigens in the host could then lead to an increased risk of SCC in SPKTRs, which could potentially affect SCC more severely than BCC, as SCCs are more antigenic cancers than are BCCs (Muchemwa *et al*, 2006). Future studies should point out whether this hypothesis is true.

The overall SCC:BCC ratio in this study was 0.79, which is lower than the ratio of 1.6 in our earlier study (Hartevelt *et al*, 1990). After the introduction of maintenance therapy with MMF instead of Aza, a decreased risk of SCC was observed, while the risk of BCC was not decreased or even possibly increased. Therefore, this change in maintenance therapy may explain, at least partly, the lower SCC:BCC ratio. The length of the follow-up period may form another explanation, as BCCs tend to occur earlier after transplantation than SCCs, but after a latent period, the cumulative incidence of SCC increases more rapidly than that of BCC.

The high collinearity of the immunosuppressive regimen, as well as HLA matching with the type of organ transplanted and the relatively limited numbers of first events, is the most important limitation of this study. The high collinearity could easily result in overfitting in the model so that the association between transplanted organ and skin cancer could disappear. The limited numbers of first events provided insufficient power, limiting the number of reliable stratified analyses.

As the risk of developing skin cancers in transplant recipients is highly increased, excessive exposure to sunlight should be avoided and use of daily sunscreen should be advised. In addition, strict control in an outpatient clinic is important for diagnosing skin cancers at an early stage, facilitating the best treatment and preventing further complications.

Materials and methods

Patients

All 208 patients who received a SPKT at the LUMC between March 1986 and January 2006 were included in this cohort study and were compared with all 1,111 KTRs transplanted in the LUMC during the same time period. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Collection of data

Data recorded for all SPKTRs and KTRs included the country of origin, the dates of the transplantations, age at transplantation, sex, and the dates of death or last follow-up visit. During the first post-transplant years, all patients with functional grafts were seen in the Department of Nephrology, LUMC. Only 88 (6.7%) patients (4 SPKTRs and 84 KTRs) were later followed up in other centers in the Netherlands. In total, 11 (0.8%) patients (1 SPKTR and 10 KTRs) were lost to follow-up, mainly because they moved to another country.

The country of origin was used as a rough estimation of the skin type. Altogether, 1,176 patients originated from the Netherlands or countries with a comparable distribution of skin type. A total of 61 patients originated from Mediterranean countries (1 from France; 2 from Israel; 2 from Iran; 2 from Iraq; 1 from Italy; 20 from Morocco; 1 from Spain; 1 from Tunisia; 26 from Turkey; and 5 from (former) Yugoslavia) and 82 from countries with a dark skin type (29 from Africa; 9 from Indonesia; 5 from other parts of Asia; and 39 from Suriname or Dutch Antilles).
Patients with skin problems were also seen and followed up at the Department of Dermatology, LUMC. Skin biopsies were routinely carried out when skin cancers were suspected. Skin cancer data were collected from the computerized oncological registry of the LUMC, the database from the department of Pathology, and from the national histological database (PALGRA). Follow-up data were collected until June 2007.

Of 1,111 KTRs, 9 recipients (5 with malignant melanoma, 2 with Kaposi's sarcoma, 1 with sweat gland carcinoma and 1 with fibrosarcoma) were present, but no SPKTR who developed skin cancers other than NMSC after transplantation. These skin cancers are not further discussed.

Immunosuppressive regimens and HLA matching

Information about the initial and maintenance immunosuppressive therapy of all patients was obtained from the Eurotransplant database. Type of induction therapy and the number and type of rejection treatments were collected from the flow sheets in the medical charts of the department of nephrology.

For SPKTRs, the initial and maintenance immunosuppressive therapy between 1986 and 1995 consisted of prednisolone (P) (7.5-10 mg/day), Aza (50-100 mg/day) and CsA (200-300mg/day). Between 1996 and 2001, almost all new patients were treated with prednisolone (7.5-10 mg/day), MMF (2,000 mg/day) and CsA (200-300 mg/day). Since 2002 the immunosuppressive treatment of all new patients consisted of prednisolone (7.5-10 mg/day), MMF (1,000-1,500 mg/day) and Tac (6-10 mg/day). In most SPKTRs, maintenance therapy was identical to initial treatment.

For KTRs, immunosuppressive treatment initially consisted of duo therapy with prednisolone and Aza, but shortly after 1986, all new KTRs were immunosuppressed with prednisolone and CsA. After 1996, triple therapy also became the treatment of choice among KTRs, whereby, initially, most new KTRs were treated with prednisolone, MMF, and CsA, and later, most new KTRs were treated with prednisolone, MMF and Tac. The target blood levels for immunosuppressive drugs were the same for the KTR group as for the SPKTR group. Of 1,111 KTRs, in 667 (60%) recipients, maintenance

therapy was identical to initial therapy. Starting in 1996, in 39 patients, CsA was switched to MMF, and in 23 patients, MMF was added to prednisolone and CsA. Details of maintenance immunosuppressive regimens, categorized according to three time periods of transplantation, for all SPKTRs and KTRs are provided in Table 3.

A total of 112 of the 208 SPKTRs received induction therapy to prevent a rejection of the graft by administration of OKT3 (24 patients), ATG (63 patients), daclizumab (23 patients) or basiliximab (2 patients). With the exception of some rare patients, induction treatments with ATG and/or OKT3 were not given to KTRs who were transplanted in the LUMC. Starting in 2000, however, induction treatment with basiliximab became common practice among KTRs.

SPKTRs and KTRs, in whom acute graft rejections were observed, were almost always initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection, a second and third rejection treatment with ATG and once more with methylprednisolone, respectively, was given. In exceptional cases, OKT3 was given when a fourth rejection treatment was needed.

To estimate the level of immunosuppression, we categorized the patients into four groups. Triple therapy instead of duo therapy and therapy with ATG or OKT3 as induction or rejection therapy were considered as factors increasing the level of immunosuppression. "Low " level of immunosuppression was defined as duo therapy without induction or rejection therapy with ATG or OKT3; "moderate" level of immunosuppression was defined as (a) triple therapy without induction or rejection therapy or (b) duo therapy with induction or rejection therapy with ATG or OKT3; "high" level of immunosuppression was defined as (a) triple therapy with induction or rejection therapy with ATG or OKT3, or (b) duo therapy with both induction and rejection therapy with ATG or OKT3; and "very high" level of immunosuppression was defined as triple therapy and both induction and rejection therapy with ATG or OKT3.

The degree of HLA mismatching for HLA-A, HLA-B, and HLA-DR antigens was assessed by counting the antigens present in the donor but absent in the recipient.

Statistical analyses

For analyses of SCCs and BCCs together, we used the term NMSC. We used all recipients with SCC (with or without BCC) and all recipients with BCC (with or without SCC) to calculate the cumulative incidence of SCCs and BCCs (Kaplan-Meier analyses). For all other analyses involving SCC and BCC, we used patients with SCCs or BCCs as first event to avoid patients with both SCCs and BCCs being used twice in our analyses. Performing our analyses on all recipients with SCC (with or without BCC) or

on all recipients with BCC (with or without SCC) did not lead to significantly different outcomes.

The initial and maintenance immunosuppressive therapies were categorized into three basic treatment groups: duo or triple therapy with Aza in any combination, duo or triple therapy with MMF in any combination, and duo therapy without Aza or MMF (i.e. a combination of prednisolone with CsA or prednisolone with Tac). If no data were available for the maintenance immunosuppressive therapy, the data of the initial immunosuppressive therapy were used. For all our analyses with immunosuppressive therapy, we used the subcategorization of maintenance therapy because the patients were, generally, exposed to this regimen for the most prolonged period of time.

Because ATG and OKT3 exert by far the highest immunosuppressive effect, induction and rejection treatments were dichotomized into those with and without ATG and/or OKT3. Because the biological effects of ATG and OKT3 are supposed to be similar before and after the transplantation, exposures to ATG and/or OKT3 as induction or rejection treatment were also combined for our analyses.

Differences between patients with and without skin cancer were analyzed by Chi-square (categorical variables) and Student's T-tests (continuous variables). Kaplan-Meier survival analyses were used to estimate the cumulative incidence of skin cancer after transplantation. Cox's proportional hazard analyses were used to calculate hazard ratios for the development of skin cancer and to adjust for potentially confounding factors. As opening dates for both analyses, we used the date of the first transplantation; as closing dates, we used the date of diagnosis of the first SCC or BCC, the date of the patient's death, the date of last follow-up, the date that they were lost to follow-up, or, if the patients were still seen in an outpatient clinic, we used the date of the end of the study (1 June 2007). The patients were not censored from analyses at graft failure. Censoring patients from analyses because of failure of the first graft did not lead to significantly different outcomes. We assessed proportionality of hazards by plotting Schoenfeld residuals for relevant covariates and by introducing interactions of relevant covariates with time in the Cox's proportional hazard model. For all statistical analyses we used SPSS version 14.0.1 (SPSS Inc, Chicago, IL).

Analytic strategy to test for confounding

First, potential risk factors for NMSC, SCC and BCC were identified with Kaplan-Meier analyses stratified for SPKTR and KTR (Supplementary Figure S1) and in multivariable Cox's proportional hazard models (Supplementary Tables S1a and S1b). Subsequently, possible confounding of the association between transplanted organ and skin cancer was tested with Kaplan-Meier analyses stratified for the potential risk factors of interest

(Supplementary Figure S2) and in multivariable Cox's proportional hazard models (Table 4). The Cox's proportional hazard analyses were initially carried out without any adjustment and subsequently with adjustments for age and sex. The hazard ratios adjusted for age and sex were further adjusted for other potentially confounding factors (Table 4). Age and sex, HLA matching and maintenance immunosuppression had the most important modulating effect on the association between transplanted organ and skin cancer, and these factors were, therefore, included in the final model. Maintenance immunosuppression, use of ATG or OKT3, and level of immunosuppression could not be included in the model together because of collinearity and overfitting.

Acknowledgements

The authors thank Jan Oosting for providing histopathological examinations and Marko Mallat, Jeroen Edelbroek, and Jan Molenaar for providing important clinical data. We are also grateful to Saskia le Cessie for her statistical advice and to Paul Douw van der Krap for his support in laying out the figure. This work was presented in part at the 11th World congress on cancers of the skin, Amsterdam, the Netherlands, 8-11 June 2007; at the 37th European Society of Dermatology Research meeting, Zurich, Switzerland, 5-8 September 2007, at the SCOPE (Skin Care in Organ Transplant Patients Europe) meeting, Venice, Italy, 10 – 12 April 2008; and at the International Investigative Dermatology meeting, Kyoto, Japan, 14-17 May 2008.

Supplementary analyses

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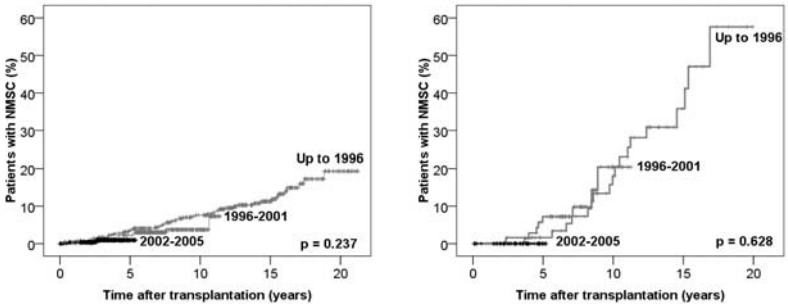
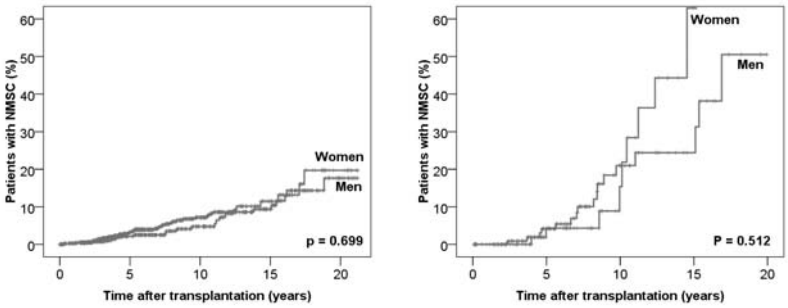
KTR SPKTR

A) Proportion of patients with NMSC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with NMSC by time after transplantation categorized by sex.



C) Proportion of patients with NMSC by time after transplantation categorized by ethnicity.

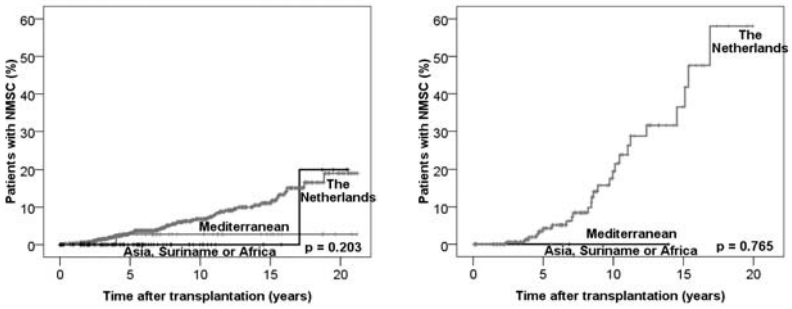
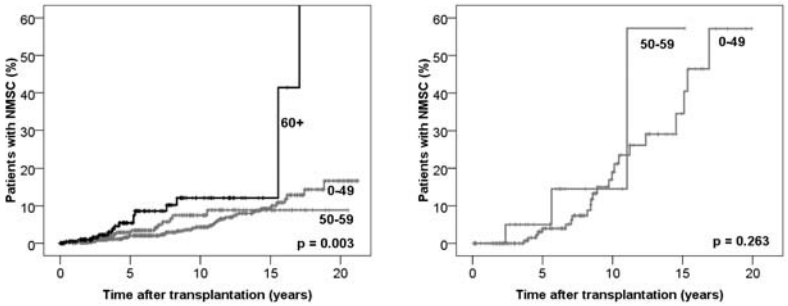


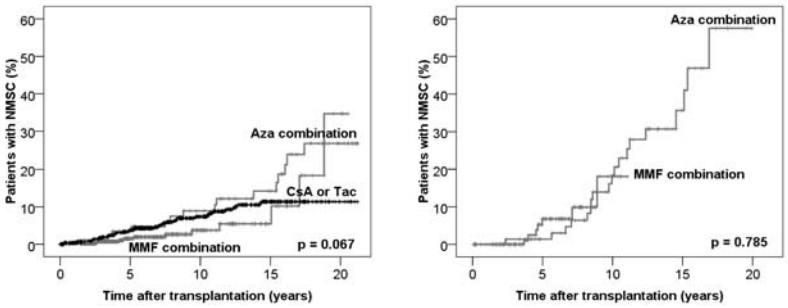
Fig S1 a-c: Risk factors for NMSC in KTR and SPKTR, page 1.

KTR SPKTR

D) Proportion of patients with NMSC by time after transplantation categorized by age.



E) Proportion of patients with NMSC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with NMSC by time after transplantation categorized by number of mismatches.

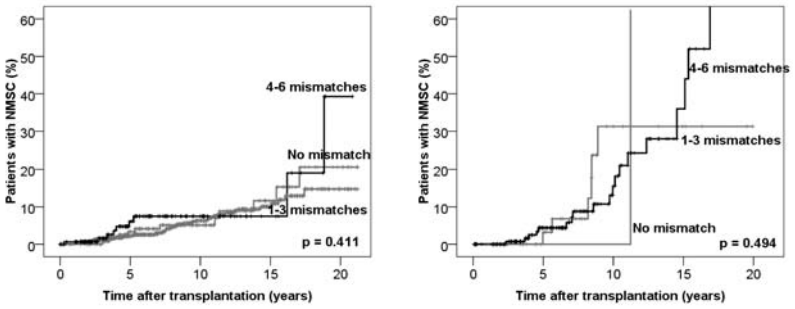
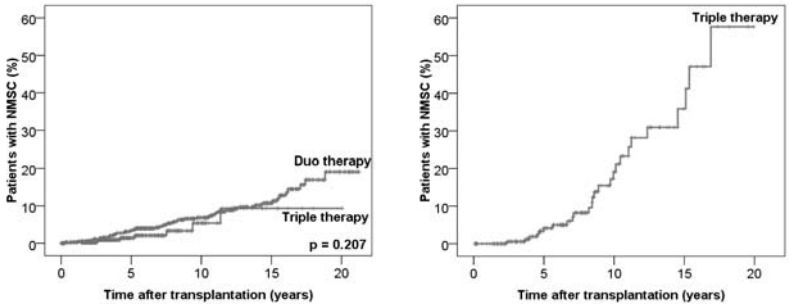


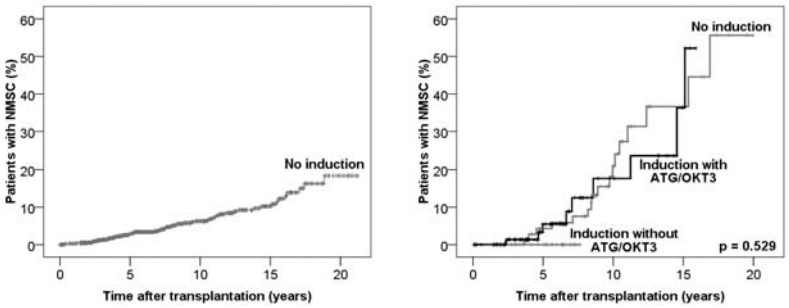
Fig S1 d-f: Risk factors for NMSC in KTR and SPKTR, page 2.

KTR SPKTR

G) Proportion of patients with NMSC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with NMSC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with NMSC by time after transplantation categorized by number of rejections.

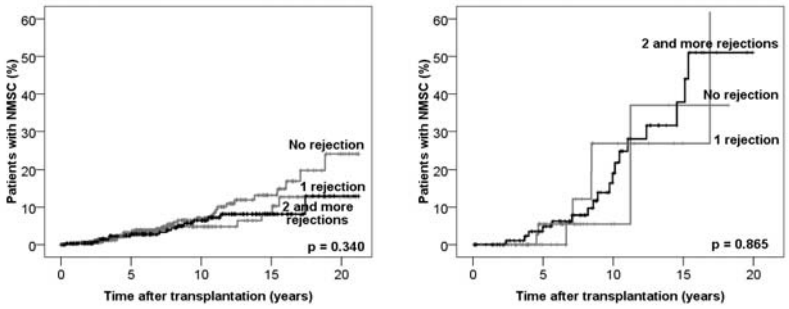
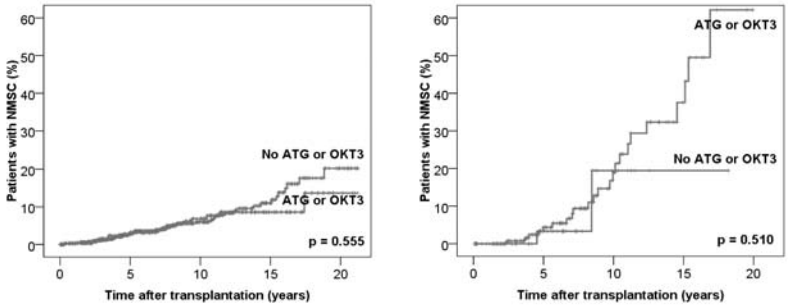


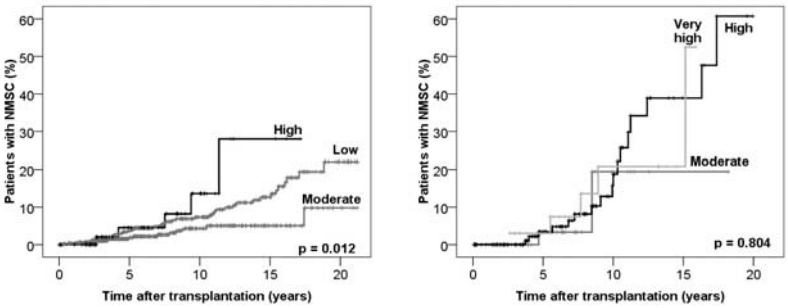
Fig S1 g-i: Risk factors for NMSC in KTR and SPKTR, page 3.

KTR SPKTR

J) Proportion of patients with NMSC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with NMSC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.



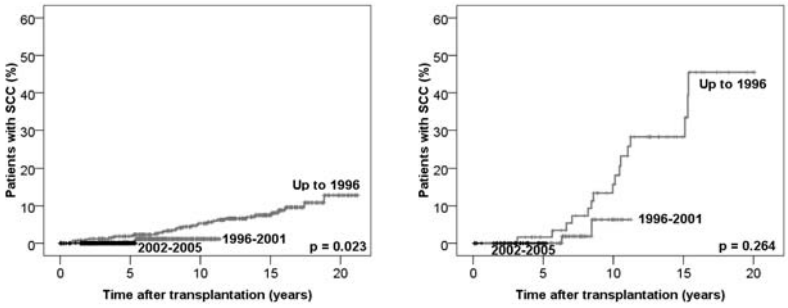
Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, NMSC = non melanoma skin cancer, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

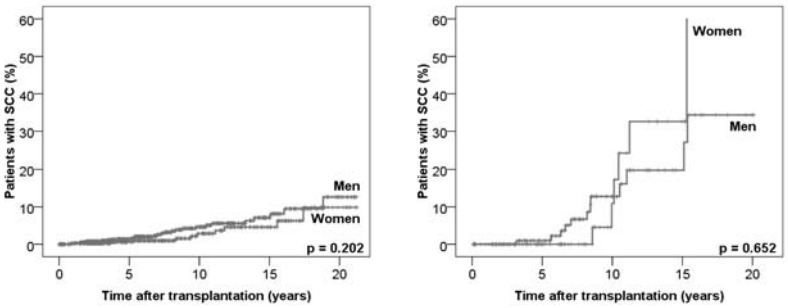
Fig S1 j-k: Risk factors for NMSC in KTR and SPKTR, page 4.

KTR SPKTR

A) Proportion of patients with SCC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with SCC by time after transplantation categorized by sex.



C) Proportion of patients with SCC by time after transplantation categorized by ethnicity.

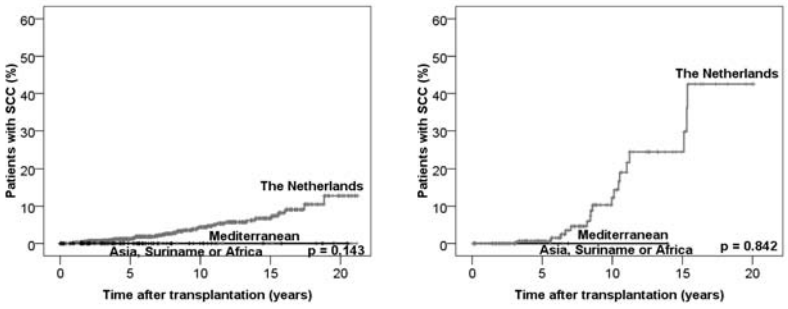
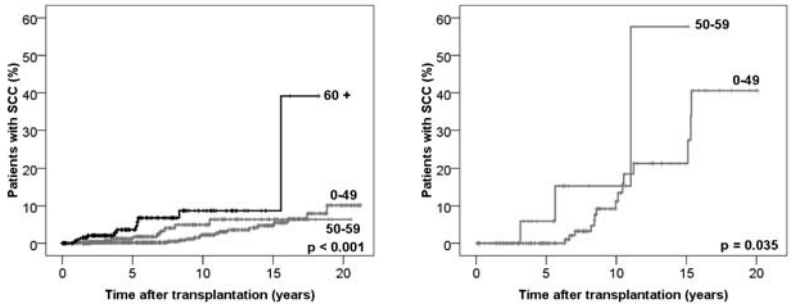


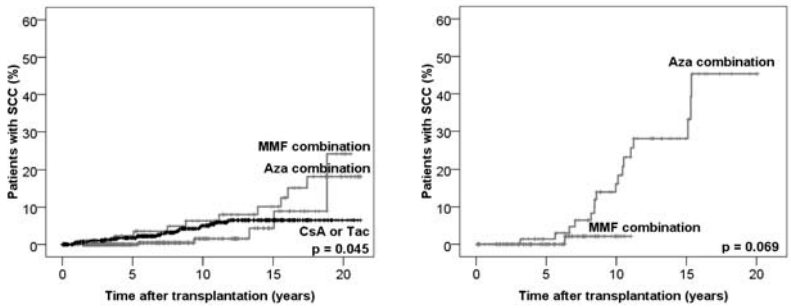
Fig S1 a-c: Risk factors for SCC in KTR and SPKTR, page 5.

KTR **SPKTR**

D) Proportion of patients with SCC by time after transplantation categorized by age.



E) Proportion of patients with SCC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with SCC by time after transplantation categorized by number of mismatches.

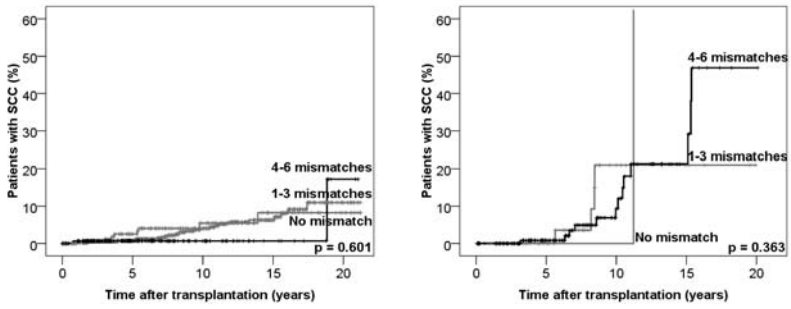
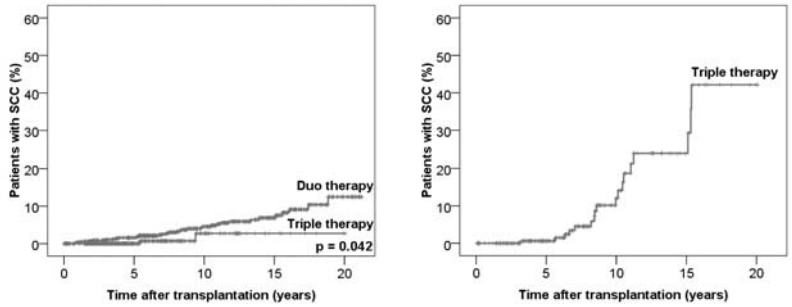


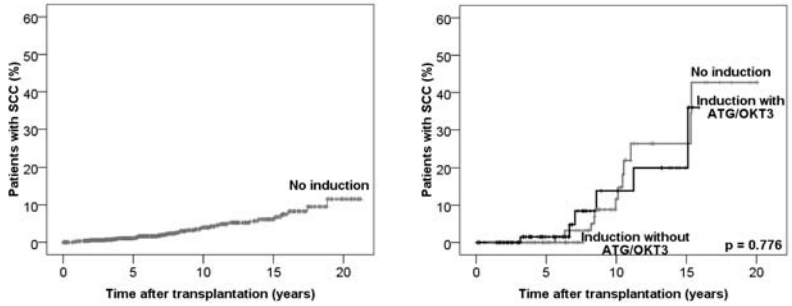
Fig S1 d-f: Risk factors for SCC in KTR and SPKTR, page 6.

KTR **SPKTR**

G) Proportion of patients with SCC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with SCC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with SCC by time after transplantation categorized by number of rejections.

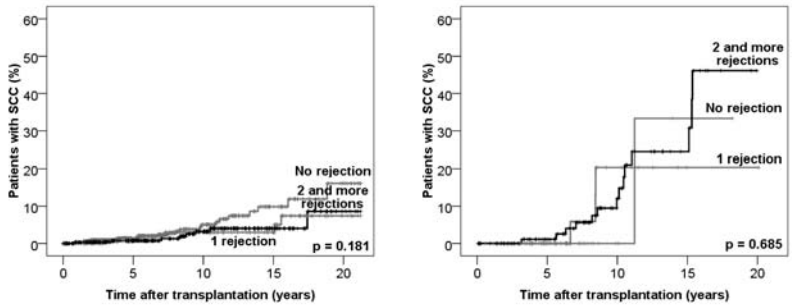
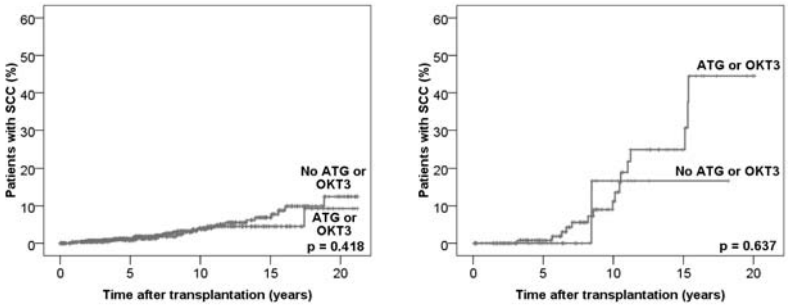


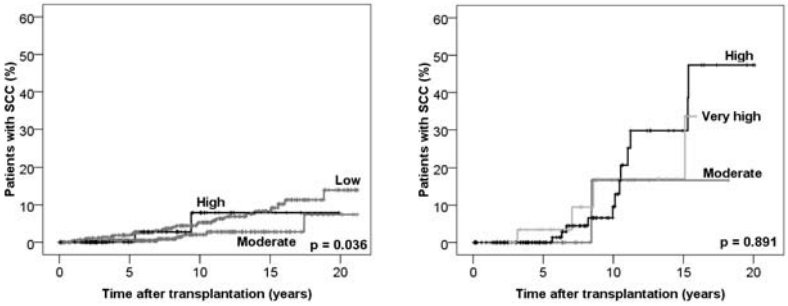
Fig S1 g-i: Risk factors for SCC in KTR and SPKTR, page 7.

KTR SPKTR

J) Proportion of patients with SCC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with SCC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.



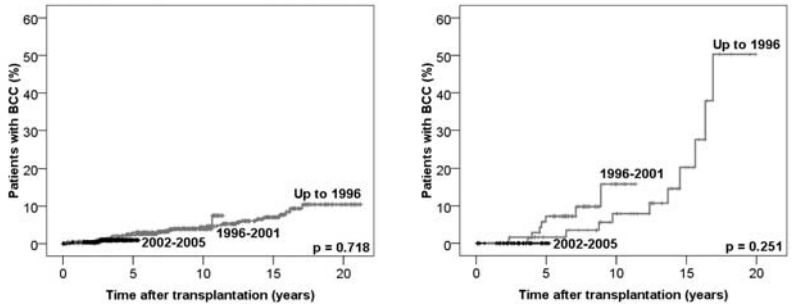
Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, SCC = squamous cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

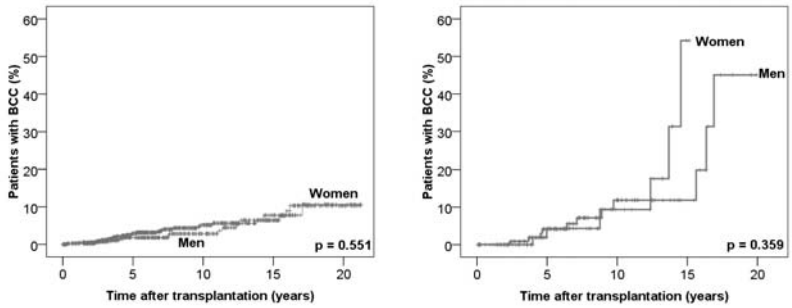
Fig S1 j-k: Risk factors for SCC in KTR and SPKTR, page 8.

KTR SPKTR

A) Proportion of patients with BCC by time after transplantation categorized by period of transplantation.



B) Proportion of patients with BCC by time after transplantation categorized by sex.



C) Proportion of patients with BCC by time after transplantation categorized by ethnicity.

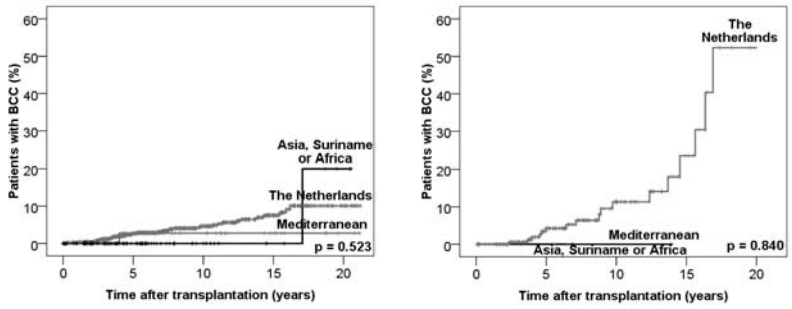
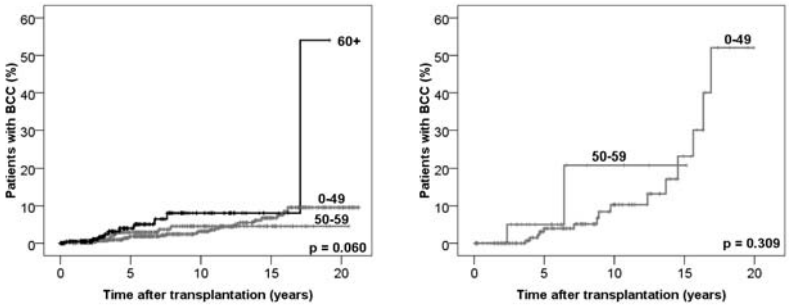


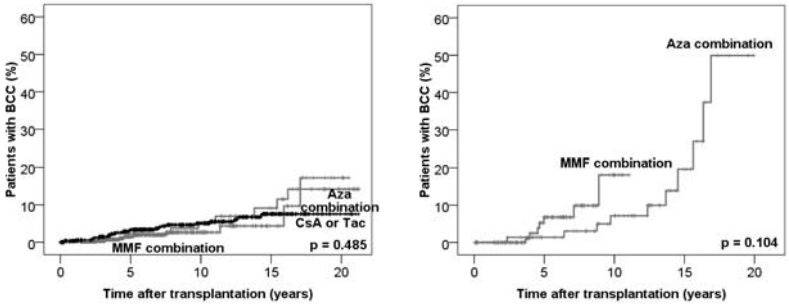
Fig S1 a-c: Risk factors for BCC in KTR and SPKTR, page 9.

KTR **SPKTR**

D) Proportion of patients with BCC by time after transplantation categorized by age.



E) Proportion of patients with BCC by time after transplantation categorized by maintenance immunosuppressive regimens.



F) Proportion of patients with BCC by time after transplantation categorized by number of mismatches.

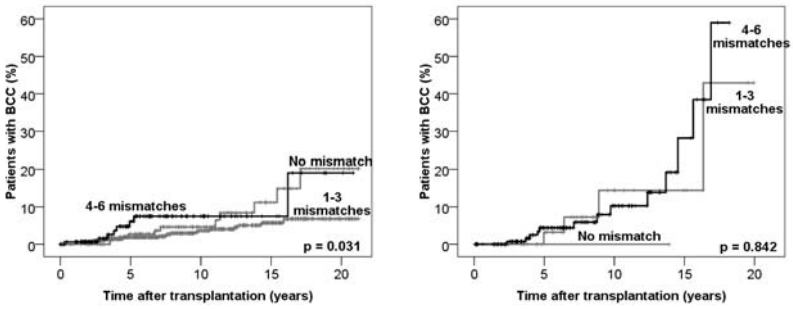
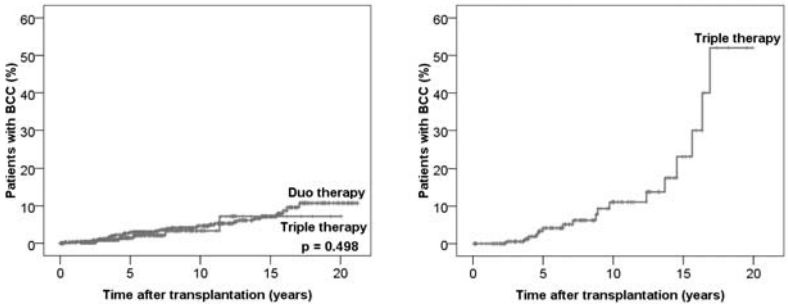


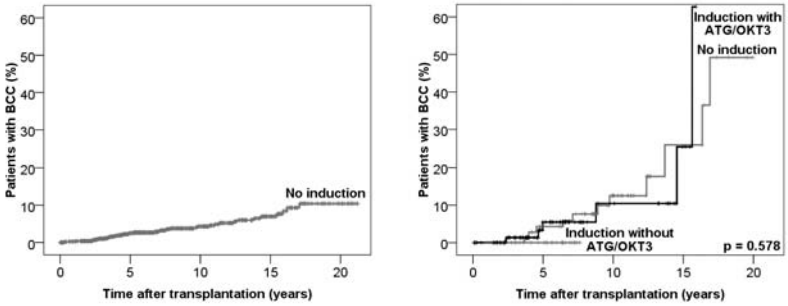
Fig S1 d-f: Risk factors for BCC in KTR and SPKTR, page 10.

KTR SPKTR

G) Proportion of patients with BCC by time after transplantation categorized by duo or triple therapy. In the SPKTR none of the patients received duo therapy.



H) Proportion of patients with BCC by time after transplantation categorized by induction therapy. In de KTR none of the patients received induction therapy.



I) Proportion of patients with BCC by time after transplantation categorized by number of rejections.

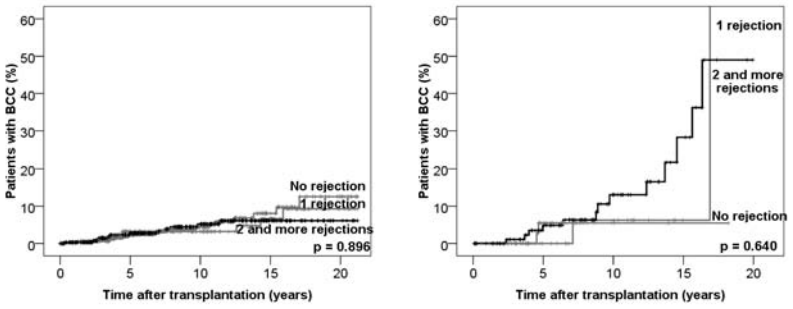
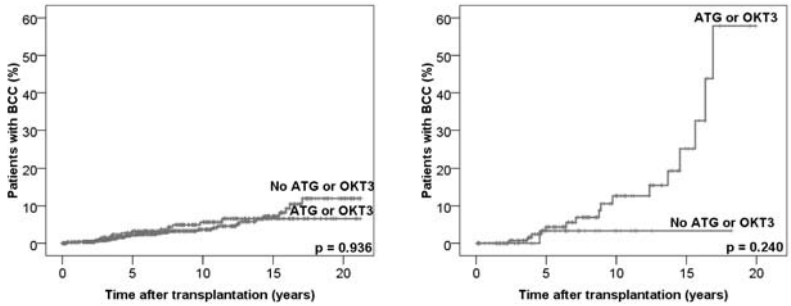


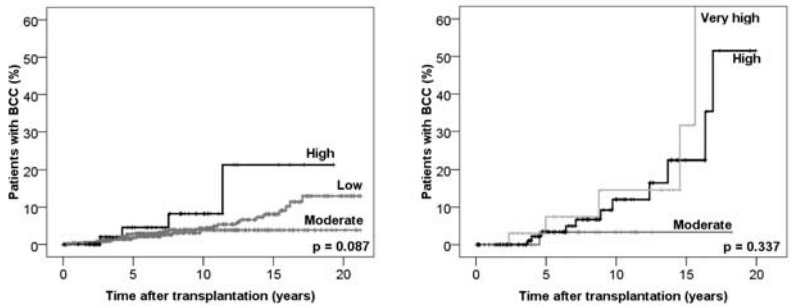
Fig S1 g-i: Risk factors for BCC in KTR and SPKTR, page 11.

KTR SPKTR

J) Proportion of patients with BCC by time after transplantation categorized by induction or rejection therapy with or without ATG or OKT3.



K) Proportion of patients with BCC by time after transplantation categorized by level of immunosuppression*; low, moderate, high, very high. In the KTR none of the patients reached a very high level of immunosuppression. In the SPKTR none of the patients received a low level of immunosuppression.

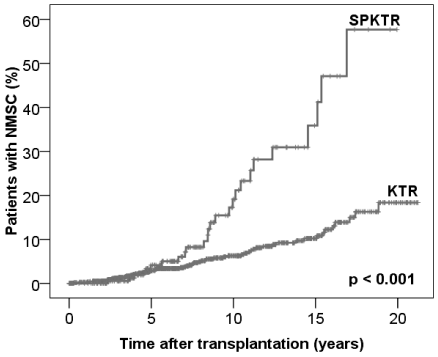


Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, BCC = basal cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

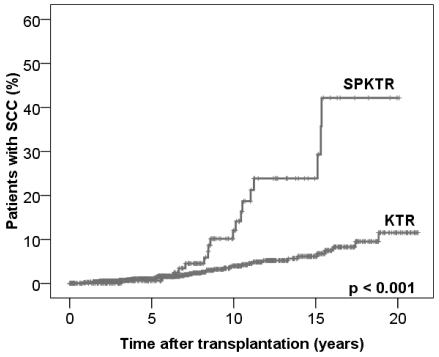
Fig S1 j-k: Risk factors for BCC in KTR and SPKTR, page 12.

Non Melanocytic Skin Cancer



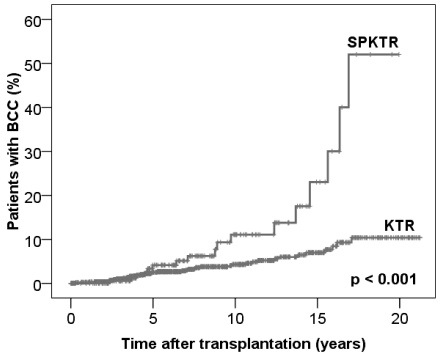
Yrs after transplantation	5	10	15
KTR	3%	6%	10%
SPKTR	4%	19%	36%

Squamous Cell Carcinoma



Yrs after transplantation	5	10	15
KTR	1%	4%	6%
SPKTR	1%	13%	25%

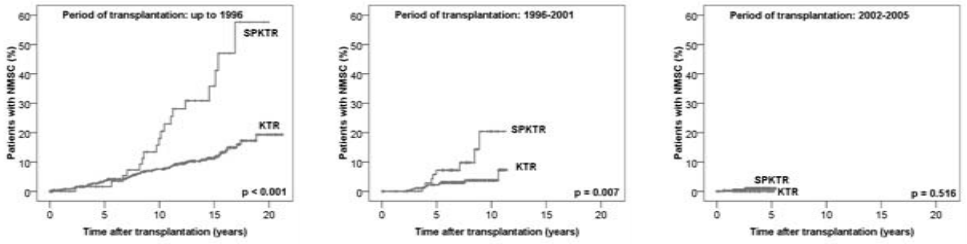
Basal Cell Carcinoma



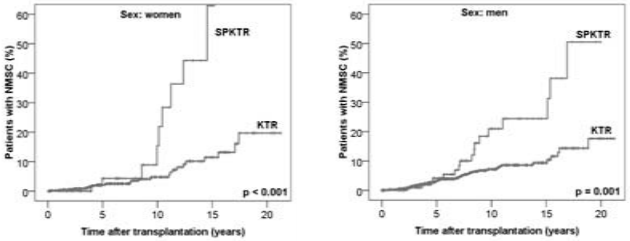
Yrs after transplantation	5	10	15
KTR	3%	4%	7%
SPKTR	4%	12%	25%

Fig S2: Risk of NMSC, SCC and BCC in SPKTR compared to KTR unstratified, page 0.

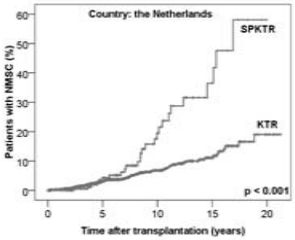
A) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by period of transplantation.



B) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 1.

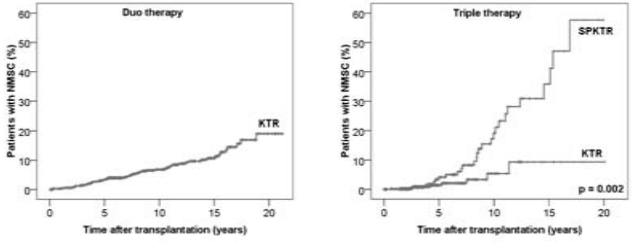
D) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by number of mismatches.

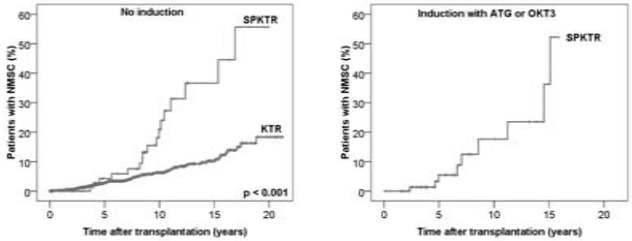
F) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 2.

G) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by number of rejections.

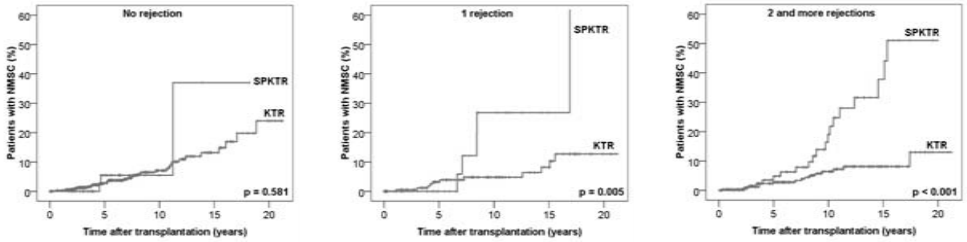
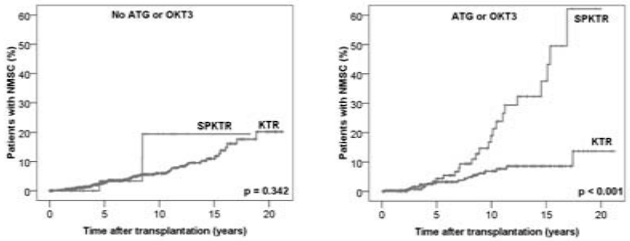
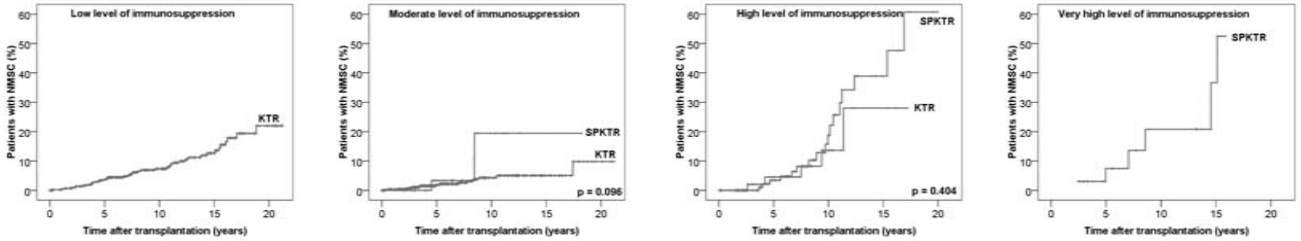


Fig S2 g-i: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 3.

J) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.



K) Proportion of KTR and SPKTR with NMSC by time after transplantation categorized by level of immunosuppression*.

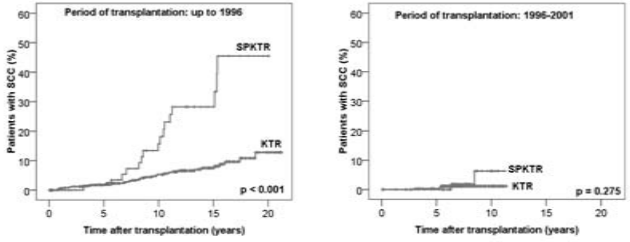


Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, NMSC = non melanoma skin cancer, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

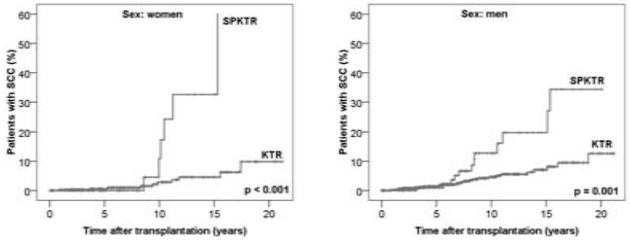
Fig S2 j-k: Risk of NMSC in SPKTR compared to KTR stratified for different factors, page 4.

A) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by period of transplantation.

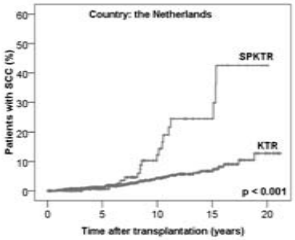


For the period of transplantation between 2002-2005 no Kaplan-Meier curve could be calculated.

B) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 5.

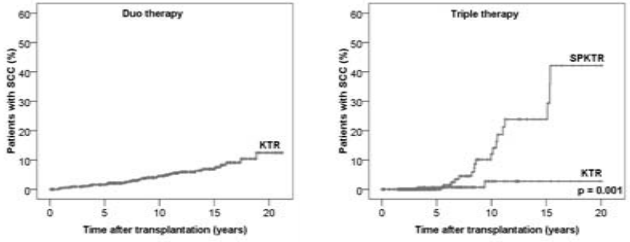
D) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by number of mismatches.

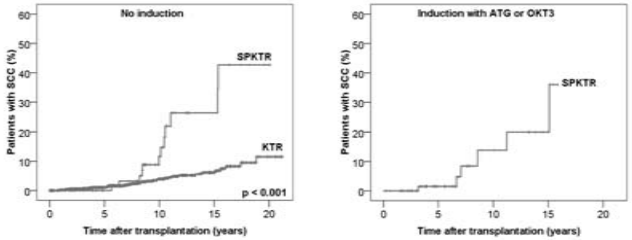
F) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 6.

G) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by number of rejections.

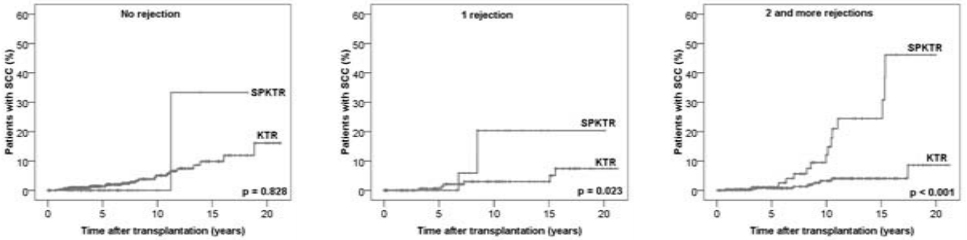


Fig S2 g-i: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 7.

J) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.

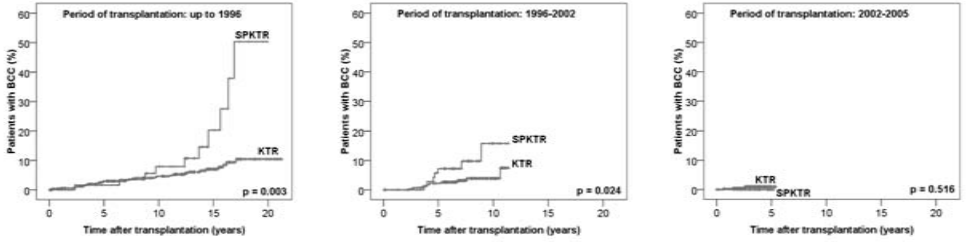
K) Proportion of KTR and SPKTR with SCC by time after transplantation categorized by level of immunosuppression*.

Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, SCC = squamous cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

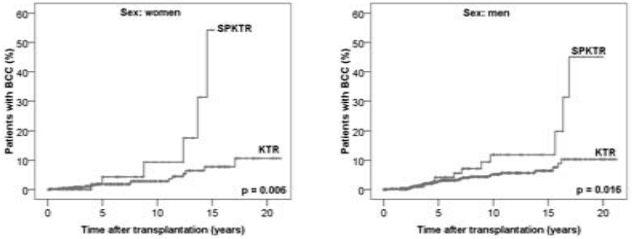
*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

Fig S2 J-k: Risk of SCC in SPKTR compared to KTR stratified for different factors, page 8.

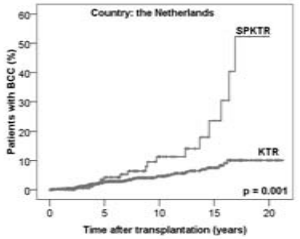
A) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by period of transplantation.



B) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by women and men.



C) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by country of origin.



For the Mediterranean and Asia, Suriname or Africa group no Kaplan-Meier curve could be calculated.

Fig S2 a-c: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 9.

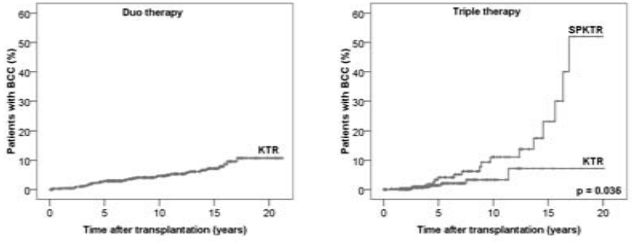
D) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by age period.

E) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by number of mismatches.

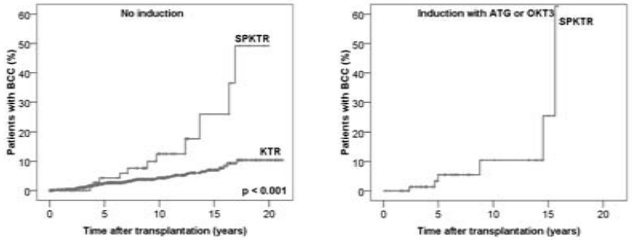
F) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by immunosuppressive therapy.

Fig S2 d-f: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 10.

G) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by duo or triple maintenance immunosuppressive therapy.



H) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by induction therapy.



For the group induction without ATG or OKT3, i.e. induction with methylprednisolone, daclizumab, basiliximab, no Kaplan-Meier curve could be calculated.

I) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by number of rejections.

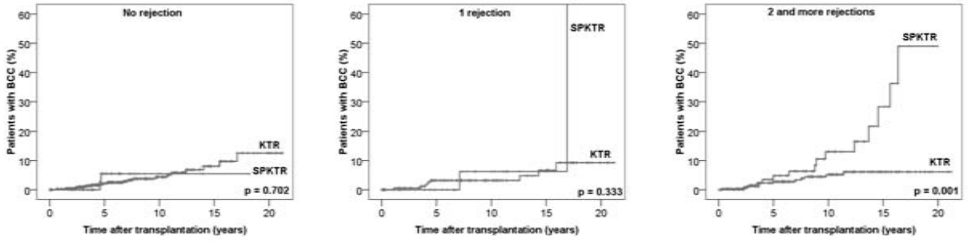
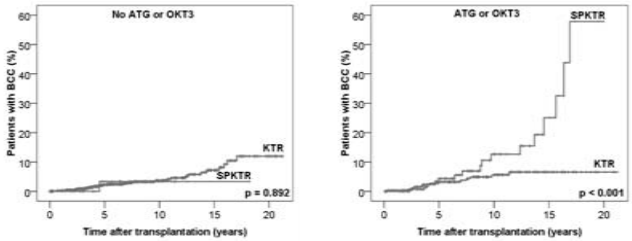
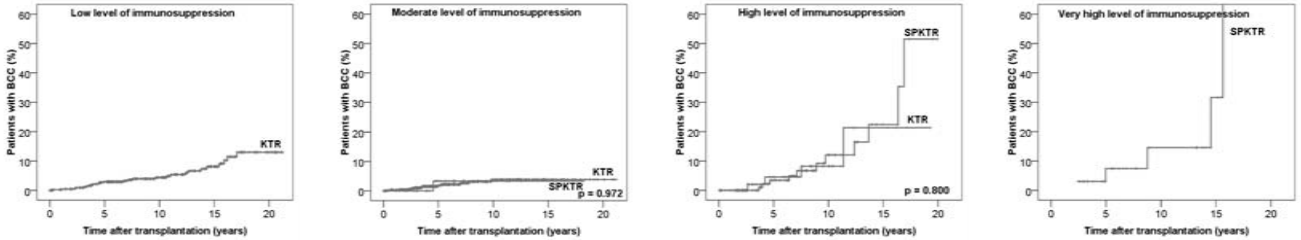


Fig S2 g-i: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 11.

J) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by induction or rejection therapy with or without OKT3 or ATG.



K) Proportion of KTR and SPKTR with BCC by time after transplantation categorized by level of immunosuppression*.



Abbreviations: KTR = kidney transplant recipients, SPKTR = simultaneous pancreas kidney transplant recipients, BCC = basal cell carcinoma, Aza = azathioprine, MMF = mycofenolatemofetil, CsA = cyclosporine, Tac = tacrolimus, ATG = antithymocyteglobulin, OKT3 = muromonab.

*Level of immunosuppression is calculated as follows: low; none of the three types of immunosuppression (triple, induction or rejection therapy) has been given, moderate; one of three types of immunosuppression (triple, induction or rejection) has been given, high; two of three types of immunosuppression (triple, induction or rejection) has been given, very high; all types of immunosuppression (triple, induction or rejection) has been given.

Fig S2 j-k: Risk of BCC in SPKTR compared to KTR stratified for different factors, page 12.

Table S1a Risk factors of skin cancer in kidney transplant recipients adjusted for age and sex using Cox proportional hazard analysis.

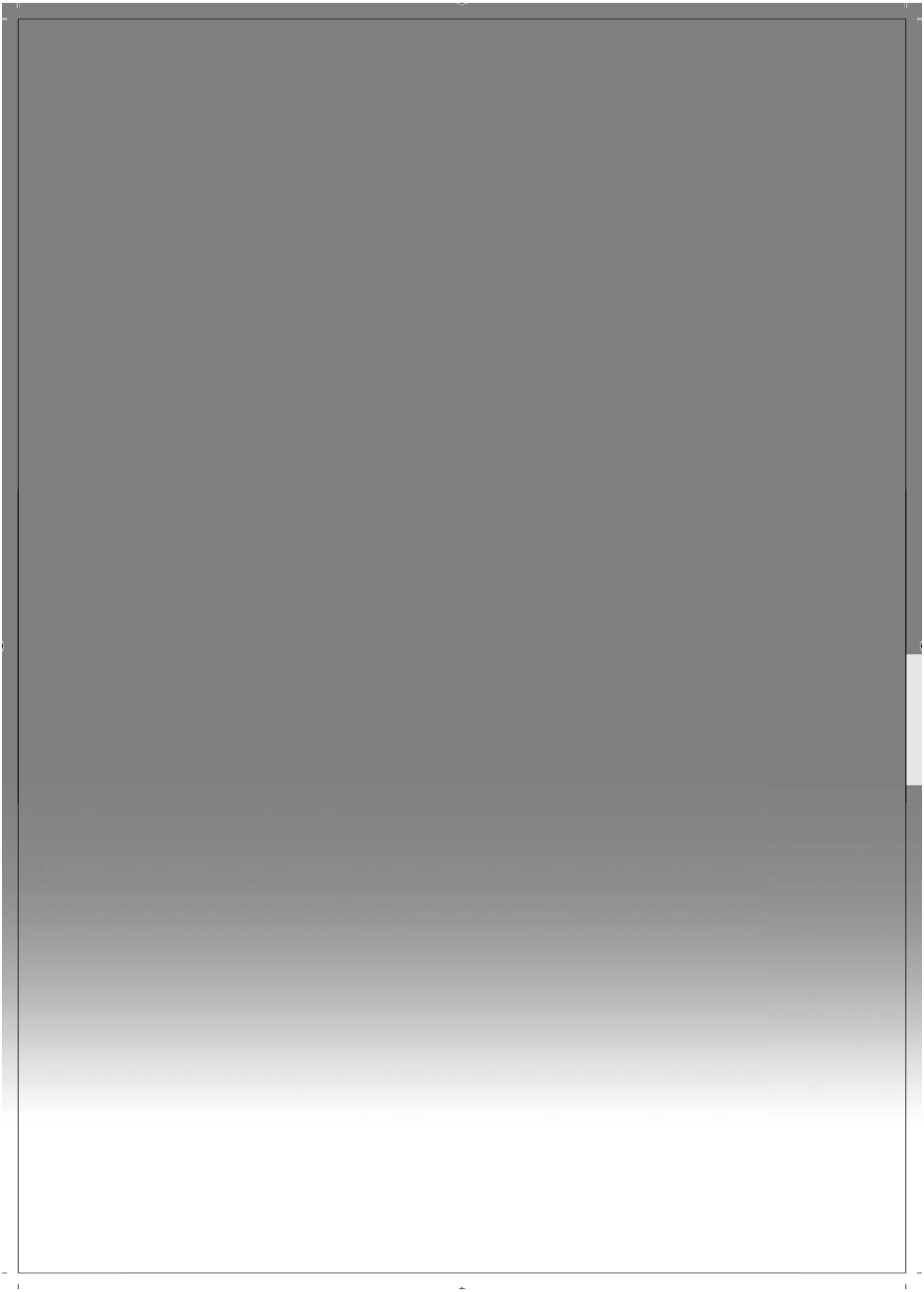
Risk factors	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
Sex			
Women	1	1	1
Men	1.2 (0.69;1.9)	1.5 (0.67;35)	0.94 (0.48;1.8)
Age			
Up to 50	1	1	1
50 – 60	1.3 (0.69;2.4)	2.2 (0.90;5.6)	0.80 (0.32;2.0)
60 and older	2.9 (1.5;5.5)	4.6 (1.7;12.5)	2.2 (0.03;5.0)
Country of origin			
Netherlands	1	1	1
Mediterranean	0.36 (0.05;2.6)	No events	0.59 (0.08;4.3)
Suriname, Africa, Asia	0.35 (0.05;2.6)	No events	0.55 (0.07;4.0)
HLA mismatching			
0	1	1	1
1-3	0.92 (0.48;1.7)	1.5 (0.51;4.3)	0.63 (0.28;1.4)
4-6	1.5 (0.63;3.6)	0.56 (0.06;5.0)	1.9 (0.70;5.1)
ATG or OKT3 as induction or rejection treatment			
No	1	1	1
Yes	0.92 (0.53;1.6)	0.84 (0.35;2.0)	0.97 (0.46;2.0)
Type of maintenance immunosuppression*			
Aza in any combination	1	1	1
MMF in any combination	0.35 (0.16;0.77)	0.15 (0.04;0.59)	0.57 (0.19;1.7)
CsA or Tac	0.53 (0.28;0.99)	0.35 (0.15;0.84)	0.71 (0.28;1.8)
Level of immunosuppression			
Low	1	1	1
Moderate	0.47 (0.26;0.86)	0.42 (0.17;1.0)	0.50 (0.23;1.1)
High or very high	1.8 (0.72;4.7)	0.95 (0.13;7.3)	2.5 (0.83;7.3)

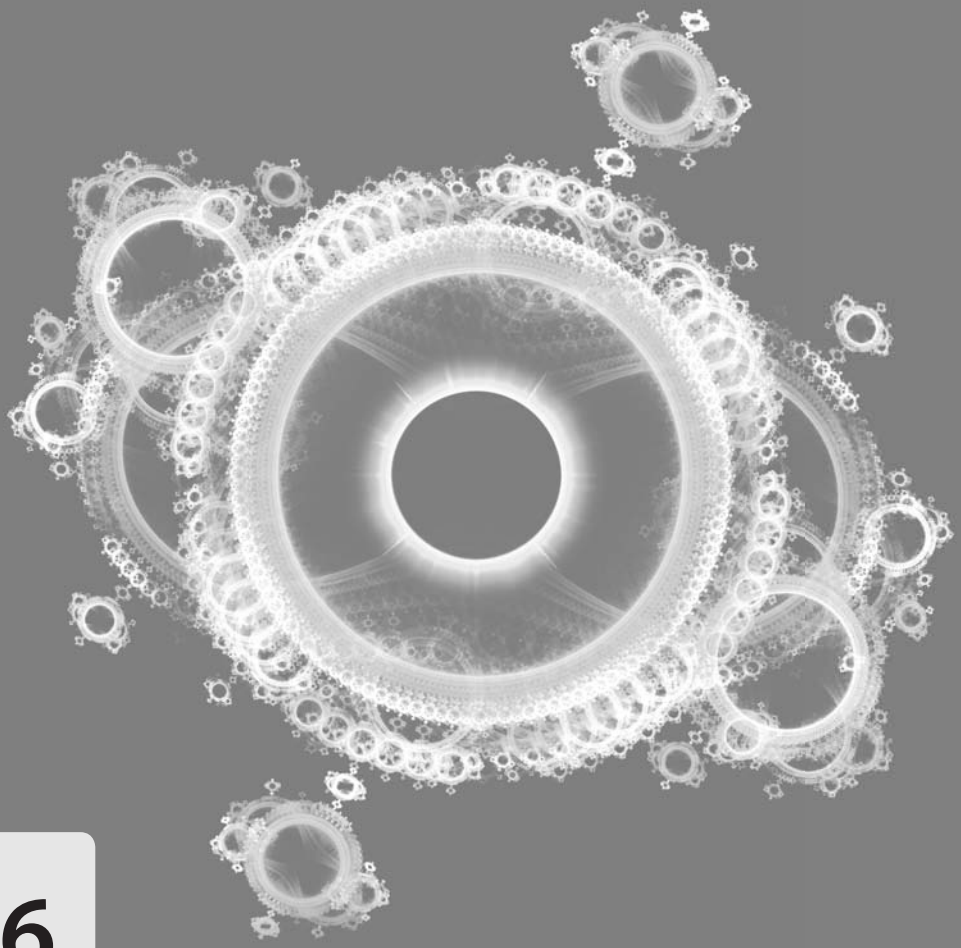
*Aza: azathioprine; MMF: mycophenolate mofetil; CsA: cyclosporine; Tac: tacrolimus.

Table S1b Risk factors of skin cancer in simultaneous pancreas kidney transplant recipients adjusted for age and sex using Cox proportional hazard analysis.

Adjustments	Non melanocytic skin cancer	Squamous-cell carcinoma as first event	Basal-cell carcinoma as first event
Sex			
Women	1	1	1
Men	0.75 (0.33;1.7)	0.64 (0.21;2.0)	0.84 (0.24;2.9)
Age at transplantation			
Up to 50	1	1	1
50 – 59	1.9 (0.56;6.5)	2.5 (0.53;11.6)	1.2 (0.15;9.8)
60 and older	No patients	No patients	No patients
Country of origin			
Netherlands	1	1	1
Mediterranean	No events	No events	No events
Suriname, Africa, Asia	No events	No events	No events
HLA mismatching			
0-3	1	1	1
4-6	0.90 (0.38;2.2)	0.65 (0.21;2.0)	1.8 (0.38;8.3)
ATG or OKT3 as induction or rejection treatment			
No	1	1	1
Yes	1.6 (0.46;5.3)	1.3 (0.27;5.8)	2.6 (0.33;20.6)
Type of maintenance immunosuppression			
Aza in any combination	1	1	1
MMF in any combination	1.1 (0.42;3.1)	No events	4.2 (0.80;22.1)
CsA or Tac	No SPKTR in this group	No SPKTR in this group	No SPKTR in this group
Level of immunosuppression			
Low or moderate	1	1	1
High	0.69 (0.16;2.9)	0.98 (0.15;6.3)	0.35 (0.03;3.6)
Very high	1.1 (0.42;2.9)	1.3 (0.34;5.1)	0.89 (0.22;3.7)

*Aza: azathioprine; MMF: mycophenolate mofetil; CsA: cyclosporine; Tac: tacrolimus.





6

Cutaneous squamous- and basal-cell carcinomas are associated with an increased risk of internal malignancies in kidney transplant recipients

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Jan N. Bouwes Bavinck

Submitted

Abstract

The aim of this study was to investigate whether the development of cutaneous squamous- and basal-cell carcinomas is associated with an increased risk of internal malignancies in kidney transplant recipients.

In a cohort study, all 1869 patients receiving a kidney transplantation between 1966 and 2006 at the Leiden University Medical Center were followed. All malignancies which had developed between 1966 and 2007 were recorded. Time-dependent Cox regression analyses were used to study the association between the development of skin cancer and internal malignancies.

Among 1869 kidney transplant recipients, 176 (9.4%) developed cutaneous squamous- and 142 (7.6%) basal-cell carcinomas. A total of 142 (7.6%) patients developed internal malignancies after transplantation. In patients with squamous-cell carcinoma the adjusted risk to develop internal malignancies was 3.5 (2.2-5.6) and for basal-cell carcinoma patients, this risk was 2.1 (1.2-3.5). Particularly, the risk to develop carcinomas of the digestive organs, lungs and male genital organs was increased.

Kidney transplant recipients with squamous- and basal-cell carcinomas have an increased risk to develop internal malignancies. [167 words]

Introduction

Kidney transplant recipients (KTR) have a significantly increased risk of malignancy [1-5]. The incidence of malignancies is 2- to 6-fold higher than in the general population [4-7]. Especially the incidences of non-melanocytic skin cancer (NMSC), comprising squamous-cell carcinoma (SCC) and basal-cell carcinoma (BCC), post-transplant lymphoma, anogenital dysplasia, thyroid cancer and Kaposi's sarcoma are increased [1;4;6;8-12]. NMSC are the most common post-transplant malignancies [9] and many KTR develop multiple malignancies [13;14].

In the general population, patients with a cutaneous SCC have a 2-fold increased risk of internal malignancies [15-17], but some studies showed no increased risk of internal malignancies, or even a slightly decreased risk, after the development of cutaneous SCC [18-20]. In the general population, the development of BCC was also associated with an increased risk of internal malignancies [15;21;22]. The other way round, internal malignancies were also associated with an increased risk of cutaneous SCC {Hemminki, 2003 2422 /id; Brennan, 2005 2420 /id}.

No previous studies have investigated the association between NMSC and internal malignancies in KTR. In this study we investigated the risk of internal malignancies after the development of cutaneous SCC or BCC in KTR.

Material and methods

Patients

We performed a cohort study of all 1869 patients who received a first kidney transplantation at the LUMC between March 1966 and January 2006. The follow-up of the patients ended arbitrarily on 1 June, 2007. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Collection of data

Data recorded for all KTR included the date of the first transplantation, age at transplantation, sex, and the dates of cancer, death or last follow-up. The main outcomes of cancer were the diagnoses of internal malignancies, cutaneous SCC and/or BCC and were collected from the computerized oncological registry of the LUMC, the database from the department of Pathology and the national histological database (PALGA) [25]. The medical charts were also hand searched for the diagnosis of cancer.

Premalignant lesions and in situ carcinomas were excluded.

The diagnoses of internal malignancies were based on the International Classification of Diseases 10th Modification Diagnoses Codes (ICD-10). Different from the ICD-10 classification we classified lip carcinomas as cutaneous SCC or BCC and not as internal malignancies.

Immunosuppressive regimens

Between 1966 and 1986, the immunosuppressive treatment of KTR in our clinic consisted of duo therapy with prednisolone and azathioprine (Aza), but shortly after 1986 all new KTR were immunosuppressed with prednisolone and cyclosporine A (CsA). From the mid 90th occasionally KTR were treated with prednisolone, mycofenolatemofetil (MMF) and CsA.

KTR, in whom acute graft rejections were observed, were generally initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection a second rejection treatment with ATG and a third rejection treatment with once more methylprednisolone were given. In exceptional cases OKT3 was given when a fourth rejection treatment was needed. With the exception of some rare patients, induction treatments with ATG and/or OKT3 were not given to KTR who were transplanted in the LUMC between 1966 and 1995.

Statistical analyses

We calculated the time on immunosuppression by adding the times between the different transplantations and subsequent rejections or until the patient was censored. If there was no rejection, we used the time between the transplantation and the end of the study or until the patient was censored (development of malignancy, last follow-up visit, or death of the patient).

For statistical analyses we used Chi-square tests for categorical variables and Student's T-tests for continuous variables. Cox proportional hazard analyses were used to calculate hazard ratios for the development of internal malignancies, SCC or BCC and to adjust for potentially confounding factors. Time-dependent Cox regression analyses were used to measure the effect of time-dependent risk factors. As opening dates for the analyses we used the date of the first transplantation; as closing dates we used the date of diagnosis of cancer, the date of the patient's death or the date of last follow up. Patients were not censored from the analyses at graft failure.

KTR who had already cancer before the first kidney transplantation or patients who were lost to follow up at the first transplantation were excluded from all analyses. P-values below 0.05 were considered significant. All statistical calculations were performed using SPSS for Windows version 16 (SPSS Inc, Chicago, IL).

Results

Baseline characteristics of the KTR

Between March 1966 and January 2006, 1906 patients received their first kidney transplant in Leiden. Thirty-seven patients had already a malignancy before the transplantation and they were excluded from further analyses. Of the remaining 1869 KTR the median age at transplantation was 43.9 years (range 3.8 – 77.5) with a median follow up of 9.2 (range 0 -39.9) years. Altogether, 176 (9.4%) had developed cutaneous SCC; 142 (7.6%) BCC and 142 (7.6%) internal malignancies, whereas 1529 (81.8%) KTR did not develop any type of cancer. A total of 88 patients developed both SCC and BCC. Cutaneous SCC and BCC were, by far, the most frequently diagnosed cancers after transplantation [13]. In a single patient, the maximum number of SCC was 68 and the maximum number of BCC was 28. In total, there were more than 1800 SCC and BCC in these patients [13]. For this study, however, only the first SCC and BCC were considered. One hundred forty-two patients developed together 151 internal malignancies, of which 112 were carcinomas, 8 leukemias, 22 lymphomas and 2 sarcomas and in 7 cases the cellular type was undefined.

In total, 29 SCC and 8 BCC of the lip had been diagnosed in 31 KTR. In 8 of these patients, SCC of the lip was the first presentation of SCC and in 7 patients BCC of the lip was the first presentation of BCC.

Risk factors of cancer

To identify possible risk factors for the development of cutaneous SCC, BCC or internal malignancies, we analyzed the influence of sex, age at the first transplantation, the years of the first transplantation, the maintenance immunosuppressive therapy and time on immunosuppression on the risk of SCC, BCC and internal malignancies (Table 1).

Patients with SCC were significantly more often male and were significantly younger at their first transplantation compared with patients without cancer (Table 1). Performing Cox proportional hazard analyses, however, older age at transplantation appeared to be a risk factor for the development of SCC, because young patients at transplantation were much longer in the follow-up than older patients, as we have

Table 1 Baseline characteristics of 1869 kidney-transplant recipients without and with different types of malignancy.

	No Malignancy	Basal-cell carcinoma	Squamous-cell carcinoma	Internal malignancy	P-value compared with no malignancy
Number of patients: N*	1529	142	176	142	
Sex: N (%)					
Female	587 (38.4)	48 (33.8)	57 (32.4)	63 (44.4)	SCC: 0.05
Male	942 (61.6)	94 (66.2)	119 (67.6)	79 (55.6)	BCC: 0.15
					IM: 0.07
Age at first transplantation (yrs)					
Median (25% - 75%)	43.8 (32.3 – 54.8)	41.7 (31.7 – 49.9)	39.1 (28.5 – 47.3)	46.1 (35.1 – 53.3)	SCC: 0.001
					BCC: 0.17
					IM: 0.04
Age at first cancer (yrs)					
Median (25% -75%)		56.0 (41.8 – 65.9)	58.5 (46.1 – 64.2)	62.3 (51.3 – 69.6)	
Years of first transplantation: N (%)					
1966-1975	169 (11.1)	32 (22.5)	54 (30.7)	22 (15.5)	SCC: <0.001
1976-1985	320 (20.9)	65 (45.8)	84 (47.7)	51 (35.9)	BCC: <0.001 IM: <0.001
1986-1995	430 (28.1)	32 (22.5)	34 (19.3)	54 (38.0)	
1996-2006	610 (39.9)	13 (9.2)	4 (2.3)	15 (10.6)	
Immunosuppressive therapy: N (%)					
Aza combination	497 (34.3)	98 (69.0)	146 (83.0)	77 (54.2)	SCC: <0.001
MMF combination	487 (33.6)	13 (9.2)	5 (2.8)	14 (9.9)	BCC: <0.001 IM: <0.001
CyA or Tac	464 (32.0)	31 (21.8)	25 (14.2)	51 (35.9)	

Time on immunosuppression (yrs)					
0 – 9	988 (64.6)	27 (19.0)	21 (11.9)	53 (37.3)	SCC: <0.001
10 – 19	367 (24.0)	48 (33.8)	64 (36.4)	62 (43.7)	BCC: <0.001 IM: <0.001
20 or more	174 (11.4)	67 (47.2)	91 (51.7)	27 (19.0)	

SCC; squamous-cell carcinoma; BCC: basal-cell carcinoma; IM: internal malignancy.
*Some patients had internal malignancy, squamous-cell carcinoma and/or basal-cell carcinoma together. This fact is reflected here by overlapping of the numbers of patients in these categories.

reported before [13]. By contrast, patients with internal malignancies were significantly older at their first transplantation than patients who did not develop cancer and they were older at the time that they developed internal malignancies compared with the time that patients developed SCC or BCC (Table 1).

As could be expected, most patients with cancer were transplanted before 1996 and, as a consequence, were more often immunosuppressed with Aza and had a longer time on immunosuppression than patients without cancer (Table 1).

Patients with cutaneous SCC or BCC are at risk for subsequent internal malignancies

Table 2 shows the distribution of patients without and with SCC or BCC prior to the development of internal malignancies. Of the KTR with internal malignancies 22.0% had developed a prior SCC, whereas in patients without internal malignancies only 7.9% had developed SCC. Adjustment for age and sex reduced the hazard ratio, suggesting that there was partially confounding by these factors for the association between SCC and internal malignancies (Table 2). Inclusion of the patients who developed SCC after internal malignancies or additional adjustment for immunosuppressive therapy did not influence the hazard ratios, importantly (data not shown).

To analyze whether patients with SCC were at increased risk for a specific type of internal malignancy, time-dependent hazard ratios were calculated for the different types of internal malignancy, separately. The hazard ratio, adjusted for age and sex, for the 32 carcinomas of the digestive organs was 4.2 (1.8-9.7), for the 21 carcinomas of the lower respiratory system 4.6 (1.5-14.5) and for the 11 carcinomas of the male genital organs 7.3 (1.7-32.5). The risks of other types

Table 2 Risk of internal malignancy in patients with prior squamous-cell carcinoma or basal-cell carcinoma.

	No internal malignancy	Internal malignancy	Non-adjusted Hazard ratio (95% CI)	Hazard ratio adjusted for age and sex (95% CI)
Squamous-cell carcinoma: N (%)				
No	1590 (92.1)	103 (78.0)	1	1
Yes	137 (7.9)	29 (22.0)	5.0 (3.1-8.0)	3.5 (2.2-5.6)
Basal-cell carcinoma: N (%)				
No	1610 (93.3)	117 (86.7)	1	1
Yes	117 (6.7)	18 (13.3)	2.8 (1.7-4.8)	2.1 (1.2-3.5)

of internal malignancy, for example of the 22 lymphomas or of the 12 carcinomas of the female genital organs were not significantly increased after the development of SCC (data not shown). The risk of internal malignancy in BCC patients was only significantly increased for the 32 carcinomas of the digestive organs, with a hazard ratio of 2.8 (1.1-6.9).

Patients with internal malignancies are not at risk for subsequent SCC or BCC

Table 3 shows the distribution of patients without and with internal malignancies prior to the development of SCC or BCC. Of the KTR with SCC, 6.8% had developed a prior internal malignancy and in KTR with BCC 5.6% had developed a prior internal malignancy, whereas in patients without SCC 6.1% had developed an internal malignancy and in patients without BCC 6.7% had developed an internal malignancy. After adjustment for sex and age the hazard ratios of developing SCC or BCC after the development of internal malignancies were not statistically significant (Table 3).

Patients with BCC are at risk for subsequent SCC, and patients with SCC are at risk for subsequent BCC

Table 4 shows the distribution of patients without and with BCC prior to the development of SCC and the other way round. Of the KTR with SCC 32.8% had developed a prior BCC and of the KTR with BCC 42.6% had developed a prior SCC. BCC patients were at a highly increased risk to develop SCC and SCC patients were at a

Table 3 Risk of squamous-cell carcinoma or basal-cell carcinoma in patients with prior internal malignancy.

	No squamous-cell carcinoma	Squamous-cell carcinoma	Non-adjusted Hazard ratio (95% CI)	Hazard ratio adjusted for age and sex (95% CI)
Internal malignancy: N (%)				
No	1590 (93.9)	137 (93.2)	1	1
Yes	103 (6.1)	10 (6.8)	2.0 (1.0-3.9)	1.6 (0.82-3.2)
	No basal-cell carcinoma	**Basal-cell carcinoma**		
Internal malignancy: N (%)				
No	1610 (93.3)	117 (94.4)	1	1
Yes	117 (6.7)	7 (5.6)	1.6 (0.69-3.6)	1.1 (0.50-2.6)

Table 4 Risk of squamous-cell carcinoma in patients with prior basal -cell carcinoma and vise versa.

	No squamous-cell carcinoma	Squamous-cell carcinoma	Non-adjusted Hazard ratio (95% CI)	Hazard ratio adjusted for age and sex (95% CI)
Basal-cell carcinoma: N (%)				
No	1635 (96.6)	92 (67.2)	1	1
Yes	58 (3.4)	45 (32.8)	10.0 (6.8-14.7)	7.9 (5.3-11.7)
	No basal-cell carcinoma	**Basal-cell carcinoma**		
Squamous-cell carcinoma: N (%)				
No	1635 (94.7)	58 (57.4)	1	1
Yes	92 (5.3)	43 (42.6)	12.1 (7.6-19.1)	9.3 (5.8-14.9)

highly increased risk to develop BCC (Table 4). Inclusion of the patients who developed BCC after SCC and patients who developed SCC after BCC or additional adjustment for immunosuppressive therapy did not influence the hazard ratios, importantly (data not shown).

Discussion

This study showed a statistically significantly increased risk of internal malignancies in KTR with a prior SCC or BCC compared with KTR without skin cancer, which could be largely attributed to an increased risk of carcinomas of the digestive organs, lungs and male genital organs. The other way round, KTR with a prior internal malignancy did not show an increased risk to develop cutaneous SCC or BCC. KTR with a prior SCC had an increased risk of BCC and those with a prior BCC an increased risk of SCC.

The 3.5 and 2.1-fold increased risks of internal malignancies after a prior SCC or BCC, respectively, are compatible with the general population, in which a 1.2-2.0-fold increased risk of internal malignancies was reported in patients with a history of SCC or BCC [15;19;26]. In our study in KTR we did not find an increased risk of SCC or BCC after the development of internal malignancies, which is in contrast with the general population [23].

An inherited predisposition of cancer, a suboptimal immune response, or lifestyle factors (smoking, sun exposure) are all possible explanations for the increased risk of internal malignancies in patients with a prior SCC or BCC. For example, the elevated rate of lung carcinoma in patients with a prior SCC, with a hazard ratio of almost 5 in our study, is suggestive for a role of smoking, which is a well-known risk factor for both lung carcinoma and cutaneous SCC [27]. However, in the general population, an association between SCC and lung cancer was also apparent after adjustment for smoking, so that other factors may play a role, as well [15].

Our finding that KTR with a prior SCC have a 3 to 4-fold increased risk of carcinoma of the digestive organs is in disagreement with the study of Grant and Tuohimaa [18;20] who showed, in the general population, no increased risk or even a slightly decreased risk of colon carcinoma in patients with a prior diagnosis of skin cancer [18;20]. They hypothesized that the increased solar ultraviolet B radiation, to which patients with NMSC are usually exposed prior to the development of skin cancer, results in higher vitamin D levels, which are though to protect, among others, against colon carcinoma [20;28]. On the other hand, Chen et al reported a 78% higher risk for colorectal carcinoma in patients with NMSC [15]. A possible explanation of this apparent different association

may be that Chen's [15] and our study were performed in countries with relatively low amounts of summertime sun exposure (north-eastern part of the United States and the Netherlands), compared to Tuohimaa et al [20] showing a reduced risk of cancer in countries with high level of sun exposure (Australia, Singapore and Spain).

A possible limitation of our study is that we did not systematically collect data of potentially confounding factors, like smoking, sun exposure, skin type, education years and body mass index, so that we cannot adjust for these factors. In another study, however, it was shown that, adjustment for these factors did not decrease the increased risk of internal malignancies in patients with prior SCC or BCC [15].

This is the first study in KTR showing an increased risk of internal malignancies, in particular carcinomas of the digestive organs, lungs and male genital organs after the development of cutaneous SCC or BCC. Both nephrologists and dermatologists should be aware of the increased risk of internal malignancies in KTR with prior skin cancers and should be extra alert when skin cancers start to develop in their patients.

Acknowledgements

The authors thank Jan Oosting for providing histopathological examinations and Marko Mallat and Jan Molenaar for providing important clinical data.

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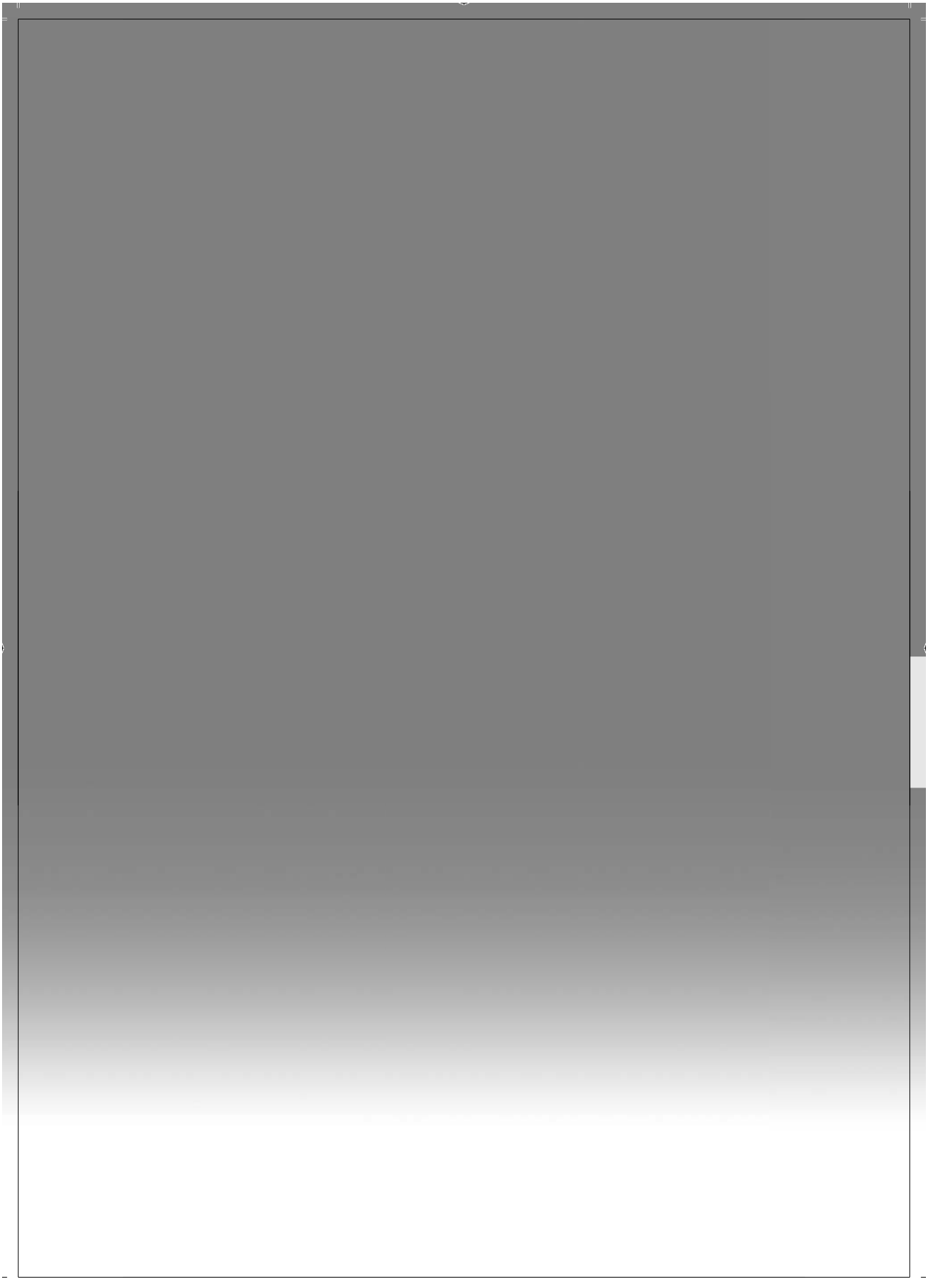

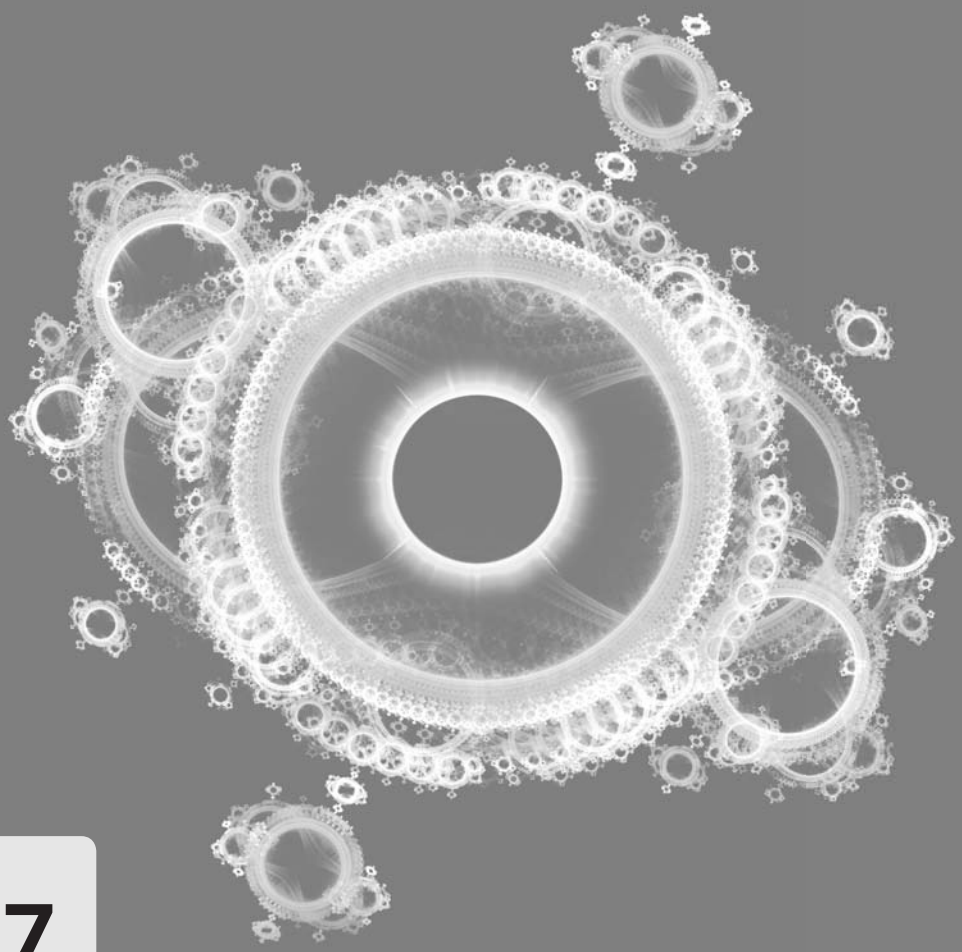

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The risk of cancer is not increased in patients with multiple kidney transplantations

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Submitted

Abstract

The aim of this study was to investigate whether the number of transplantations, as a marker of the graft rejection status of the patient, is associated with an increased risk of malignancies. In a cohort study, 1213 patients, receiving a kidney transplantation between 1966 and 1995 at the Leiden University Medical Center, were analyzed. All cutaneous squamous cell carcinoma and internal malignancies, which had developed between 1966 and 2007, were recorded. The influence of number of transplantations, age, sex and time on immunosuppression on the risk of squamous cell carcinoma and internal malignancies was investigated by time-dependent multivariate Cox's proportional hazard models. Of the 1213 kidney transplant recipients, 319 received a second kidney, 78 a third; 13 of them a fourth and 4 of them a fifth transplantation. After adjustment for potentially confounding factors, including age, sex and years on immunosuppressive therapy we did not detect an increased risk of cancer in patients with multiple transplantations. On the contrary, patients with three or more transplantations had a 1.6-fold decreased risk of squamous cell carcinomas and a 3.6-fold decreased risk of internal malignancies. We conclude that kidney transplant recipients with three or more transplantations do not have an increased risk of cutaneous squamous cell carcinoma and internal malignancies. (207 words)

Introduction

Kidney transplant recipients have an increased risk of malignancies of which, in the Caucasian population, cutaneous squamous cell carcinoma is the most common one (1-9). Chronic immunosuppressive therapy is the major risk factor for the development of abundant numbers of malignancies in kidney transplant recipients (2,6,10-12) since immunosuppression disrupts antitumor immunosurveillance and anti-viral activity. In addition, the higher the cumulative level of immunosuppression, the higher the risk to develop malignancies (6,13-15).

A well-known risk factor for graft rejection is circulating human leucocyte antigen (HLA)-antibodies, induced by pregnancy, blood infusion or previous transplantations (16-20). Other factors negatively influencing the outcome of kidney transplantation include young recipient age (young age is associated with a relatively high state of immunologic responsiveness to alloantigens), older donors, recipients of African-American origin, prolonged cold ischemia time, and systemic diseases such as diabetes (16-20). There are also studies suggesting that genetic polymorphisms play a role in the clinical outcome of transplantation, although evidence is lacking and large prospective studies are needed to show clinical applicability (21,22).

Patients who are rejecting their grafts are treated with high doses of immunosuppressive therapy, including treatments with methylprednisolone, anti-thymocyte globulin (ATG) and muronomab-CD3 (OKT3) (23,24). One could speculate that these high doses of immunosuppressive rejection therapy lead to a low activated immune response and an increased risk of malignancies. There is only one study showing that rejection treatments are associated with a higher risk of squamous cell carcinomas (25). Using high serum creatinine levels at 1 year after transplantation as a measure of graft rejection, Bordea et al showed that patients with high serum creatinine levels had a higher risk of developing skin cancer (2). They postulated that patients with a high serum creatinine level had maintained higher levels of immunosuppression to prevent rejection, which may have led to a higher risk of skin cancer (2). Bordea et al, however, did not observe an increased incidence of skin cancer in patients receiving additional immunosuppression in the form of rejection treatments with ATG and OKT3, which is in line with several other studies (2,10,11,26,27).

The aim of this study was to investigate whether the number of transplantations, which we used as a measure of graft loss, is associated with an increased risk of post-transplant malignancies.

Results

Characteristics of all patients and dropouts

Between March 1966 and 31 December 1996, 1246 patients received their first kidney transplant in Leiden. Twenty-six patients had already cancer before the transplantation and 7 patients were lost to follow up immediately after transplantation. These patients were excluded from further analyses. Of the remaining 1213 patients, 237 received the first transplantation between 1966 and 1975; 454 between 1976 and 1985 and 522 between 1986 and 1995. In total, 817 patients lost the first graft and 319 of them received a second kidney. Altogether, 78 kidney transplant recipients received a third; 13 of them a fourth and 4 of them a fifth transplantation. Of all 1213 kidney transplant recipients, 752 (62.0%) died, with a median time after transplantation to death of 10.2 years.

Baseline characteristics of kidney transplant recipients with one, two or three or more transplantations

The baseline characteristics of kidney transplant recipients with one, two or three or more transplantations are depicted in Table 1. Almost 50% of the patients with only one transplantation were transplanted before 1986, whereas 75% of the patients with two transplantations and almost 90% of the patients with three or more transplantations were transplanted before 1986 (Table 1). As a result, the follow-up time was statistically significantly longer in the patients with three or more transplantations compared to the patients with two transplantations, whereas the latter patients were followed longer than the patients with only one transplantation (Table 1).

The sex distribution did not differ statistically significantly between the three groups (Table 1). There was, however, a statistically significant association between the number of transplantations and the age at the first transplantation: with increasing number of transplantations, the age at the first transplantation was decreasing (Table 1). During time, the age of the patients at the first transplantation was significantly increasing, but this was less obvious for patients with two or three or more transplantations (Table 1).

Since most of the patients with two or more transplantations were transplanted before 1986, they were initially more frequently immunosuppressed with Aza, whereas patients with only one transplantation were more frequently immunosuppressed with CsA or Tac (Table 1). Despite important differences in follow-up time, the time on immunosuppression was not statistically significantly different between the three groups with 12.6, 13.1 and 14.4 years on immunosuppression in patients with one, two or three or more transplantations, respectively (Table 1).

Table 1 Baseline characteristics of the kidney-transplant patients with 1, 2 or 3 or more transplantations.

	Only 1 transplantation	2 transplantations	3 or more transplantations
Number of patients: N	**894**	**241**	**78**
Years of first transplantation: N (%)			
1966-1975	151 (16.9)	61 (25.3)	25 (32.1)
1976-1985	292 (32.7)	118 (49.0)	44 (56.4)
1986-1995	451 (50.4)	62 (25.7)	9 (11.5)
Follow-up until last rejection (yrs)#			
Median (25% - 75%)	12.6 (4.9 – 19.2)	16.8 (10.1 – 23.5)	20.4 (14.6 – 26.1)
0 – 1 years: N (%)	154 (17.2)	12 (5.0)	0
2 – 7 years	150 (16.8)	38 (15.8)	10 (12.8)
8 – 12 years	158 (17.7)	30 (12.4)	8 (10.3)
13 – 17 years	183 (20.5)	51 (21.2)	9 (11.5)
18 – 22 years	101 (11.3)	45 (18.7)	17 (21.8)
23 or more years	148 (16.6)	65 (27.0)	34 (43.6)
Sex: N (%)			
Female	331 (37.0)	101 (41.9)	28 (35.9)
Male	563 (63.0)	140 (58.1)	50 (64.1)
Age at first transplantation (yrs)			
Median (25% - 75%)	43.4 (32.2 – 52.5)	34.0 (23.9 – 43.3)	25.6 (18.3 – 33.9)
Immunosuppressive therapy: N (%)			
Aza combination	473 (52.9)	169 (70.4)	64 (82.1)
MMF combination	59 (6.6)	8 (3.3)	0
CyA or Tac	362 (40.5)	63 (26.3)	14 (17.9)
Time on immunosuppression (yrs)			
Median (25% - 75%)	12.6 (4.9 – 19.2)	13.1 (5.9 – 20.0)	14.4 (6.8 – 21.6)
0 – 9 years	356 (39.8)	86 (35.7)	27 (34.6)
10 – 19 years	328 (36.7)	95 (39.4)	27 (34.6)
20 or more years	210 (23.5)	60 (24.9)	24 (30.8)

Aza, azathioprine; MMF, mycophenolatemofetil; CsA, cyclosporine A; Tac, tacrolimus
#Follow-up until last rejection or end of follow-up or death (when there was a functioning graft at the time of death).

Distribution of squamous cell carcinomas and internal malignancies by number of transplantations

In total, 301 (24.8%) of the 1213 kidney transplant recipients developed any type of malignancy. During the follow up period 169 (13.9%) out of 1213 patients developed at least one cutaneous squamous cell carcinoma. Although there were fewer patients with three or more transplantations who developed squamous cell carcinomas, if they developed a squamous cell carcinoma, this occurred longer after the transplantation, but at a younger age, which probably reflects their younger age at the first transplantation (Table 2). Altogether, 120 (9.9%) out of the 1213 kidney transplant recipients developed an internal malignancy. Only 2 (2.6%) patients with three or more transplantations developed an internal malignancy and only 7 (8.9%) developed a cutaneous squamous cell carcinoma, which is much lower compared to patients with only 1 or 2 transplantations (Table 2). Kidney transplant recipients with 4 or 5 transplantations did not develop any malignancies. Details of the 9 patients with 3 transplantations and malignancies are provided in Table 3.

Table 2 Distribution of cancer among the kidney-transplant patients with 1, 2 or 3 or more transplantations.

	Only 1 transplantation	2 transplantations	3 or more transplantations
Number of patients: N	**894**	**241**	**78**
Number of patients with SCC: N (%)	123 (13.8)	39 (16.2)	7 (8.9)
Age at first SCC (yrs) Median (25% - 75%)	54.2 (45.9-60.2)	52.7 (42.7-58.3)	43.1 (32.8-43.9)
Time from first transplantation to first SCC (yrs) Median (25% - 75%)	11.8 (7.5-17.5)	14.9 (10.9-20.3)	17.7 (13.0-22.5)
Number of patients with internal malignancy: N (%)	94 (10.5)	24 (10.0)	2 (2.6)
Age at internal malignancy (yrs) Median (25% - 75%)	58.1 (50.4-62.2)	52.3 (39.3-61.9)	35.1 and 58.8
Time from first transplantation to internal malignancy (yrs) Median (25% - 75%)	9.9 (4.0-15.4)	11.8 (6.9-17.8)	4.0 and 10.8

SCC, squamous cell carcinoma

Table 3 Characteristics of the patients with 3 transplantations and malignancies.

Patient	Year of birth	Years of transplantation	Years of rejection	Year of internal malignancy	Year of first squamous cell carcinoma	Year of Death
1 male	1931	1979 1980 1987	1980 1984	1990		1990
2 male	1950	1982 1982 2000	1982 1997	1986		
3 female	1940	1969 1976 1987	1971 1976		1982	1992
4 male	1955	1974 1975 1976	1974 1975		1998	
5 male	1966	1981 1982 1984	1982 1984		1999	
6 male	1960	1984 1988 1994	1988 1992		2004	
7 male	1960	1981 1983 1985	1983 1983		2003	
8 female	1963	1983 1994 2002	1987 2000		1995	
9 male	1932	1975 1976 1984	1975 1983		1989	1990

Risk factors of squamous cell carcinomas and internal malignancies

To identify possible risk factors for the development of cutaneous squamous cell carcinomas and internal malignancies, we analyzed the influence of time period of the first transplantation, sex, the age at the first transplantation, the number of transplantations, the maintenance immunosuppressive therapy and time on immuno-

suppression on the risk of squamous cell carcinomas and internal malignancies (Table 4). Patients with squamous cell carcinoma were more frequently transplanted between 1966 and 1975 and had a longer follow-up time (Table 4). After adjustment for follow-up time, they were significantly older at the first transplantation. Similarly, patients with internal malignancies were also significantly older at the first transplantation. Both squamous cell carcinoma and internal malignancy patients were more frequently immunosuppressed with Aza and had a longer time on immunosuppression than patients without cancer (Table 4).

The risk of squamous cell carcinomas and internal malignancies by number of transplantations

Figure 1A shows the cumulative incidence of squamous cell carcinomas and figure 1D of internal malignancies by number of transplantations. The cumulative incidence of squamous cell carcinoma was 8%, 22% and 40%, respectively, 10, 20, and 30 years after transplantation in patients with only one transplantation, in contrast with 1%, 11%, and 14% in patients with three or more transplantations. For internal malignancies the cumulative incidences were 7%, 15%, and 23% at the same time points, whereas the cumulative incidence of internal malignancies only reached 3% for patients with three or more transplantations (Figure 1D). Figures 1A and 1D show that three or more transplantations are not associated with an increased risk of squamous cell carcinomas and internal malignancies in kidney transplantation recipients. These figures rather suggest a decreased risk of these malignancies in patients with three or more transplantations. Patients with 3 and more transplantations were significantly younger at their first transplantation than patients with only 1 transplantation. Figures 1B and 1C show the cumulative incidence of squamous cell carcinomas and figure 1E and 1F of internal malignancies by number of transplantations stratified for patients who were younger or older than 40 years at their first transplantation. In the stratified analyses, transplant recipients with 3 and more transplantations still have a decreased risk of malignancies, but the differences are less significant, indicating confounding by age.

Table 5 shows the non-adjusted and adjusted time dependent hazard ratios of developing cutaneous squamous cell carcinomas and internal malignancies by number of transplantations. In the non-adjusted analyses, we found a significantly decreased risk of squamous cell carcinomas in patients with three or more transplantations with a hazard of 0.36 (0.15-0.89). Adjustment for age raised the hazard ratio to 0.47 (0.19-1.16), also suggesting confounding by age. Additional time-dependent adjustment for years on immunosuppression raised the hazard further to 0.62 (0.23-1.6), indicating additional confounding by number of years on immunosuppression.

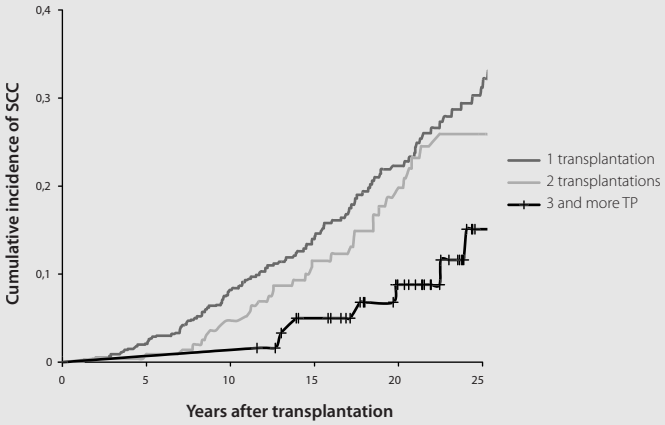
Table 4 Risk factors of cancer in the kidney-transplant recipients.

	No Cancer	Squamous cell carcinoma	Internal malignancy	P-value
Number of patients: N*	**912**	**169**	**120**	
Years of first transplantation: N (%)				
1966-1975	168 (18.4)	53 (31.4)	20 (16.7)	SCC: <0.001
1976-1985	315 (34.5)	84 (49.7)	51 (42.5)	NCM: 0.44
1986-1995	429 (47.1)	32 (18.9)	49 (40.8)	
Follow-up until last rejection (yrs)				
Median (25% - 75%)	12.4 (4.2-19.5)	22.7 (15.4-27.9)	14.3 (9.1-19.7)	SCC: <0.001
0 – 1 year: N (%)	159 (17.4)	0 (0)	5 (4.2)	NCM: <0.001
2 – 7 years	168 (18.4)	7 (4.1)	22 (18.3)	
8 – 12 years	152 (16.7)	16 (9.5)	21 (17.5)	
13 – 17 years	176 (19.3)	36 (21.3)	34 (28.3)	
18 – 22 years	116 (12.7)	29 (17.2)	19 (15.8)	
23 or more years	141 (15.5)	81 (47.9)	19 (15.8)	
Sex: N (%)				
Female	346 (37.9)	57 (33.7)	53 (44.2)	SCC: 0.13
Male	566 (62.1)	112 (66.3)	67 (55.8)	NCM: 0.08
Age at first transplantation (yrs)				
Median (25% - 75%)	39.6 (28.5 – 50.5)	38.8 (27.8 – 46.7)	45.2 (34.7 – 51.8)	SCC: 0.20
				NCM: <0.001
Number of transplantations: N (%)				
1	670 (73.5)	123 (72.8)	94 (78.3)	SCC: 0.267
2	175 (19.2)	39 (23.1)	24 (20.0)	NCM: 0.08
3 or more	67 (7.3)	7 (4.1)	2 (1.7)	
Immunosuppressive therapy: N (%)				
Aza combination	500 (54.9)	134 (79.3)	75 (62.5)	SCC: <0.001
MMF combination	56 (6.1)	5 (3.0)	1 (0.8)	NCM: 0.06
CsA or Tac	355 (39.0)	30 (17.8)	44 (36.7)	
Time on immunosuppression (yrs)				
Median (25% - 75%)	11.5 (3.3-17.5)	21.0 (14.1-27.9)	13.3 (8.1-18.8)	SCC: <0.001
0 – 9 years	412 (45.2)	15 (8.9)	36 (30.0)	NCM: 0.02
10 – 19 years	326 (35.7)	64 (37.9)	58 (48.3)	
20 or more years	174 (19.1)	90 (53.2)	26 (21.7)	

Aza, azathioprine; MMF, mycophenolatemofetil; CsA, cyclosporine A; Tac, tacrolimus
*Some patients had both internal malignancy and squamous-cell carcinoma. This fact is reflected here by overlapping of the numbers of patients in these categories.

Figure 1 Cumulative incidence of squamous cell carcinoma by number of transplantations (Panel A), and stratified for patients younger than 40 years (Panel B)...

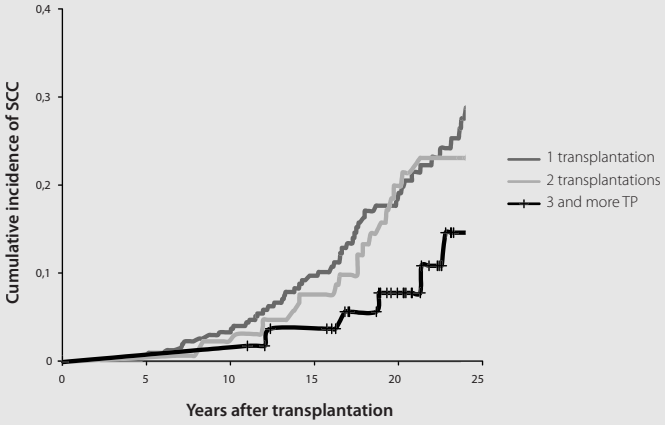
A All patients



Number of patients at risk

1 transplantation	894	652	499	304	165	7
2 transplantations	241	202	174	120	76	3
3 and more TP	78	78	78	56	43	21

B Patients younger than 40 years at transplantation

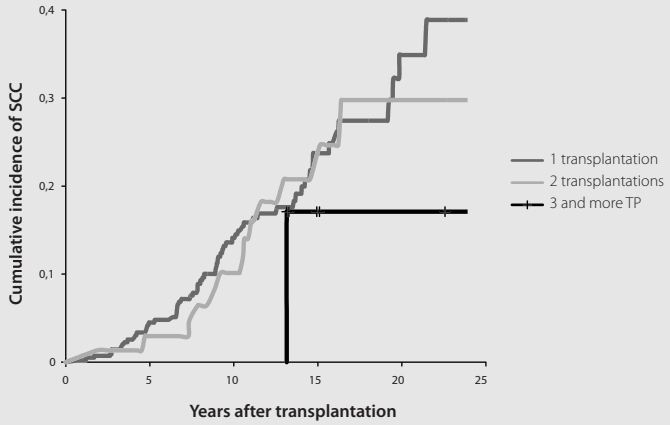


Number of patients at risk

1 transplantation	375	308	282	207	129	65
2 transplantations	160	160	124	97	67	34
3 and more TP	67	67	67	52	41	20

Figure 1 ...and patients of 40 years and older at transplantation (Panel C). Cumulative incidence of internal malignancy by number of transplantations (Panel D),...

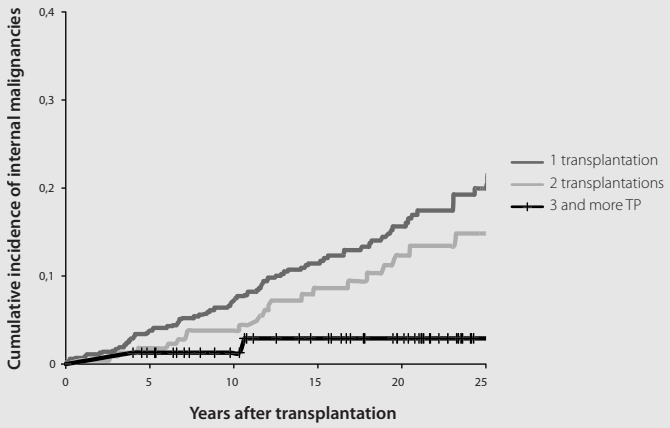
C Patients of 40 years and older at transplantation



Number of patients at risk

1 transplantation	519	344	217	97	36	9
2 transplantations	81	63	50	23	9	1
3 and more TP	11	11	11	4	2	1

D All patients

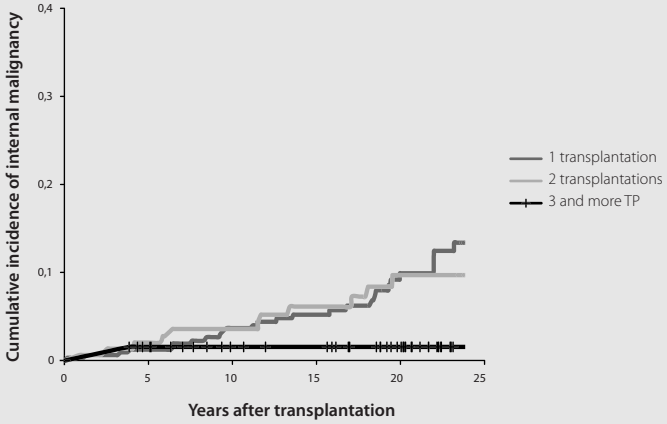


Number of patients at risk

1 transplantation	894	651	517	330	203	106
2 transplantations	241	202	178	130	86	43
3 and more TP	78	73	64	57	46	23

Figure 1 ...and stratified for patients younger than 40 years (Panel E) and patients of 40 years and older at transplantation (Panel F).

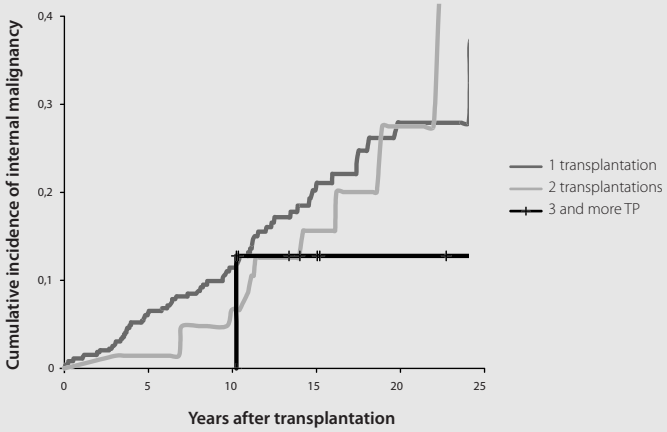
E Patients younger than 40 years at transplantation



Number of patients at risk

1 transplantation	375	307	283	221	156	89
2 transplantations	160	137	124	102	76	41
3 and more TP	67	63	56	53	44	22

F Patients of 40 years and older at transplantation



Number of patients at risk

1 transplantation	519	344	234	109	47	17
2 transplantations	81	65	54	28	10	2
3 and more TP	11	11	11	4	2	1

Table 5 Risk of squamous cell carcinoma and internal malignancies in kidney transplant recipients with adjustments for potentially confounding factors using time dependent Cox proportional hazard analyses.

Adjustments for:	Squamous cell carcinoma	Internal malignancy
No adjustments		
1 transplantation	1.0	1.0
2 transplantations	0.78 (0.52-1.2)	0.87 (0.53-1.4)
3 or more transplantations	0.36 (0.15-0.89)	0.15 (0.02-1.1)
Age and sex		
1 transplantation	1.0	1.0
2 transplantations	0.92 (0.61-1.4)	1.1 (0.67-1.9)
3 or more transplantations	0.47 (0.19-1.16)	0.27 (0.03-2.0)
Age, sex and years on immunosuppression		
1 transplantation	1.0	1.0
2 transplantations	1.05 (0.67-1.6)	1.1 (0.63-2.1)
3 or more transplantations	0.62 (0.23-1.6)	0.28 (0.04-2.3)

Both in the non-adjusted and adjusted analyses, the risk of internal malignancies was substantially decreased in patients with three or more transplantations, but this was based on only 2 occurrences of internal malignancies in the 78 patients with three or more transplantations. Because of the low power of these analyses, statistical significance was not reached and this result should, therefore, be repeated in a larger study.

Other potentially confounding factors like HLA mismatching and the level of HLA-antigen responses did not influence the hazard ratios for the development of cutaneous squamous cell carcinoma and internal malignancies, importantly (data not shown).

Discussion

In the present study we rejected the hypothesis that patients with multiple kidney transplantations may have an increased risk of squamous cell carcinomas or internal malignancies. On the contrary, we found that, after adjustment for age, sex and duration of immunosuppressive therapy, patients with three or more kidney transplantations

had a 1.6 fold decreased risk of cutaneous squamous cell carcinomas and a 3.6 fold decreased risk of internal malignancies compared with patients receiving only one kidney transplant, but these associations did not reach statistical significance.

We conclude that high doses of immunosuppressive rejection therapy with ATG and/or OKT3, which are used to treat patients who are rejecting their grafts, are not associated with an increased risk of malignancies. We hypothesize that graft loss is an indication of a high state of immunologic responsiveness to allogeneic HLA molecules in patients who are repeatedly experiencing graft losses, which may lead to a more effective (cross-reactive) immune response against malignancies, resulting in a decreased risk of malignancies. This high state of immunologic responsiveness may override the potentially increased cancer risk induced by high doses of immunosuppressive therapy for the treatment of graft rejection. This is in line with several other studies showing no association between rejection treatments and the risk of cutaneous squamous cell carcinomas (2,10,11,26,27).

Increasing age (2,11,28) and increasing number of years on immunosuppression (2,3,5,11,28-30) are well-known risk factors for malignancies. A higher state of immunologic responsiveness to allogeneic HLA molecules has not been identified as a protective factor against malignancies. Heterologous immunity is a term used to describe the partial immunity (or altered immunopathology) that occurs in response to a pathogen if the host has been previously infected or immunized with an unrelated pathogen (31). Similarly, T cells induced by viral exposure and specific for a viral peptide in the context of self-HLA may cross-react with allogeneic HLA molecules, which implies that memory T cells can be present specific for HLA antigens, toward which the patient has never been exposed. Recent data show that this type of heterologous immunity is very common (32). Thus, the viral infection can cause an increased alloreactivity to allogeneic HLA molecules which limits the induction of immunologic tolerance to the graft.

The other side of the coin is that immunologic tolerance to mismatched HLA antigens in patients with well-functioning grafts may result in a diminished immune response to viruses. We have shown that simultaneous pancreas kidney transplant recipients have an increased risk of squamous cell carcinomas compared to kidney transplant recipients (10). We speculated that the transplanted pancreas may have induced tolerance against an additional set of allo-peptides in the HLA antigens of the donor. An increased cross-reactive tolerance against squamous cell carcinomas -associated antigens in the host could have lead to an increased risk of squamous cell carcinomas in simultaneous pancreas kidney transplant recipients (10). The association between the number of graft losses and the risk of cancer may be based on such a

mechanism. We speculate that in patients who have lost several transplants, a higher state of immunologic responsiveness to allogeneic HLA molecules leads to crossreactive T cell responses to malignancy-associated antigens in the host explaining the decreased rate of malignancy in the patients with multiple transplantations despite earlier exposure to high doses of immunosuppressive therapy for the treatment of graft rejection (33).

This study was subjected to several methodological challenges. The power of the study may have been not large enough to exclude a type-2 error, i.e. there may be a positive association between the number of transplantations and the risk of malignancies, but the study was too small to pick this up. The consistently negative association between the number of transplantations and the risk of malignancies in the adjusted and stratified analyses and the statistically significant negative associations in the non-adjusted analyses, however, provide strong arguments against a possible type-2 error. Furthermore, the patients with 3 and more transplantations were significantly younger at their first transplantation than patients with only 1 transplantation and substantial amounts of time were spent off immunosuppression, so that confounding by age and time on immunosuppression formed another serious problem. It is possible that the observed negative association between the number of transplantations and the risk of malignancies can be completely attributed to the younger age of the patients with 3 and more transplantations at their first transplantation and the relatively shorter time on immunosuppression, but after adjustment for these factors there was still a negative association between these factors and malignancies which could be attributed to a higher state of immunologic responsiveness or even other possible residual confounding factors that were not tested for.

In summary, this study rejected the hypothesis that patients with multiple kidney transplantations may have an increased risk of cutaneous squamous cell carcinomas or internal malignancies and gives some support to the hypothesis that a higher state of immunologic responsiveness to allogeneic HLA molecules in these patients, as measured by a higher risk of graft losses, may protect against malignancies. More studies should be performed, however, to confirm this hypothesis.

Materials and Methods

Patients

All patients who received a first kidney transplantation at the Leiden University Medical Center (LUMC) between March 1966, when the kidney transplantation program started, and 31 December 1995, allowing for sufficient follow-up time to develop malignancies, were included in this cohort study. The follow-up of the patients ended arbitrarily on 1 June, 2007. The study adhered to the Declaration of Helsinki Principles and the medical ethical committee of the LUMC had approved the study design.

Collection of data

Data recorded for each patient included gender, dates of birth, death or last follow-up and the dates of the first transplantation and, if appropriate, the dates of the first and subsequent graft losses and subsequent re-transplantations.

We separately analyzed the risk of cutaneous squamous cell carcinomas and internal malignancies. Squamous cell carcinoma and internal malignancy data were collected from the computerized oncological registry of the LUMC, the database from the department op Pathology and the national histological database (PALGA) (10).
The Eurotransplant database provided information about the HLA types of the recipients and donors and the level of panel reactive antibodies (%PRA). The degree of HLA mismatching for HLA-A, B, and DR antigens was assessed by counting the antigens present in the donor but absent in the recipient.

Immunosuppressive regimens

Between 1966 and 1986, the immunosuppressive treatment of kidney transplant recipients in our clinic consisted of duo therapy with prednisolone and azathioprine (Aza), but shortly after 1986 all new kidney transplant recipients were immuno-suppressed with prednisolone and cyclosporine A (CsA). From the mid 90th occasionally kidney transplant recipients were treated with prednisolone, mycofenolatemofetil (MMF) and CsA.

Kidney transplant recipients, in whom acute graft rejections were observed, were generally initially treated with methylprednisolone. When this therapy was not sufficient to prevent further rejection a second rejection treatment with ATG and a third rejection treatment with once more methylprednisolone were given. In exceptional cases OKT3 was given when a fourth rejection treatment was needed. With the exception of some rare patients, induction treatments with ATG and/or OKT3

were not given to kidney transplant recipients who were transplanted in the LUMC between 1966 and 1995.

Statistical analysis

The patients were categorized into patients with one, two or three or more transplantations. We calculated the time on immunosuppression by adding the times between the different transplantations and subsequent graft losses or until the patient was censored. If there was no graft loss, we used the time between the transplantation and the end of the study or until the patient was censored (development of malignancy, last follow-up visit, or death of the patient).

For statistical analyses, we used Chi-square tests for categorical variables and Student's T-tests for continuous variables. Kaplan Meier survival analyses were used to estimate the cumulative incidence of squamous cell carcinomas and internal malignancies stratified by number of transplantation. Time-dependent Cox proportional hazard analyses were used to calculate hazard ratios for the development of squamous cell carcinomas and internal malignancies, to adjust for potentially confounding factors and to measure the effect of the time-dependent risk factors (number of transplantations and time on immunosuppression). P-values below 0.05 were considered statistically significant. As opening dates for the latter analyses we used the date of the first transplantation; as closing dates we used the first occurrence of the following mile stones: a) date of diagnosis of the first squamous cell carcinomas or internal malignancies, b) the date of the last graft loss, c) the date of the patient's death, d) the date of last follow up or e) if the patients were still followed in the outpatient clinic, the date of the end of the study (June 1, 2007).

Kidney transplant recipients who had already cancer before the first kidney transplantation or patients who were lost to follow up at the first transplantation were excluded from all analyses. All statistical calculations were performed using SPSS for Windows version 16 (SPSS Inc, Chicago, IL).

Acknowledgements

The authors thank Jan Oosting for providing histopathological examinations and Marko Mallat and Jan Molenaar for providing important clinical data. We are also grateful to Theo Stijnen for his statistical advice.

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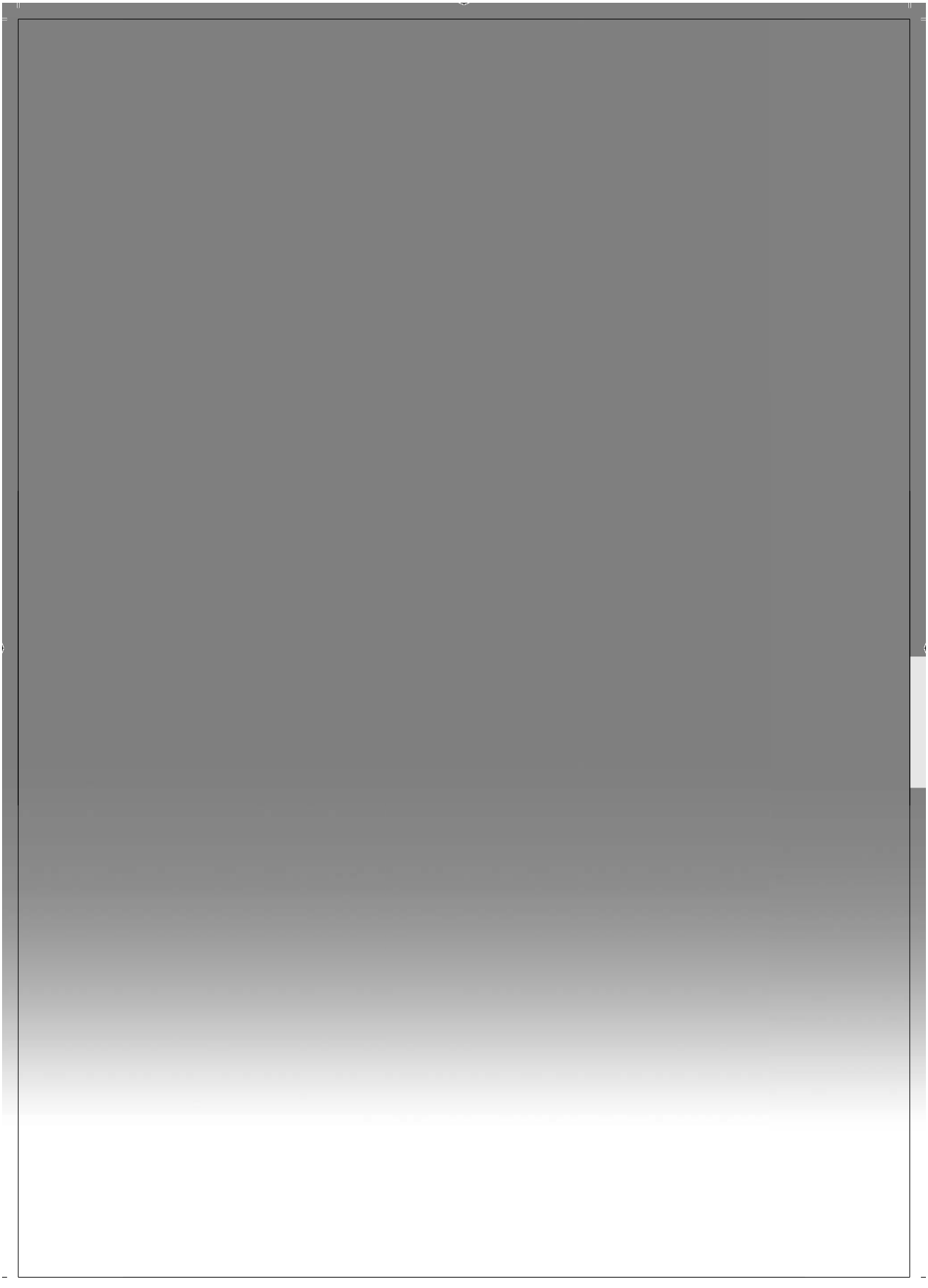
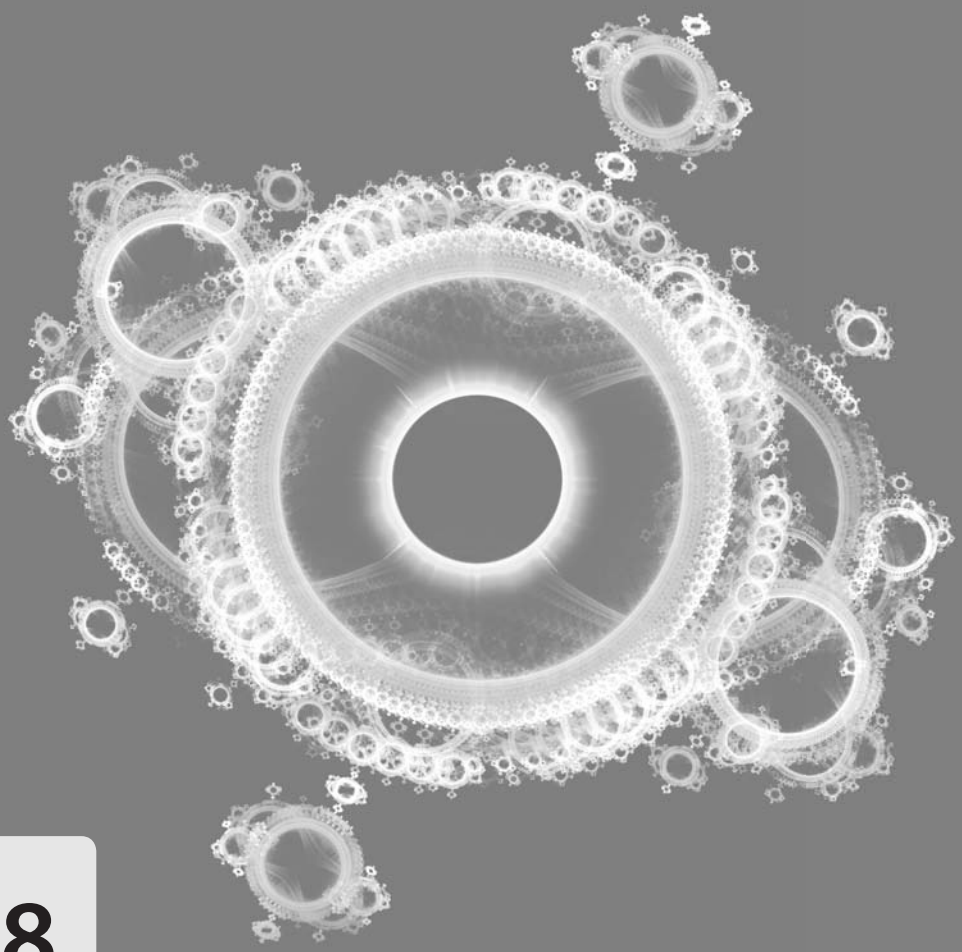




8

Summary and discussion

Introduction

The risk of (skin) cancer and other skin diseases is highly increased in organ transplant recipients (OTR) who are kept on immune suppressive drugs to prevent graft rejection (Table 1). This thesis dealt with the epidemiologic aspects and risk factors for cancer, focused on cutaneous squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) and other skin diseases in this group of patients. The studies presented in **Chapter 2-4 and 6** focused on *descriptive epidemiology*, to characterize and report both the pattern and frequency of cancer and skin diseases in OTR. **Chapter 5 and 7** were mainly based on *analytic epidemiology*, where we searched for new risk factors for cancer in OTR. Both descriptive and analytic components together contribute to increasing our understanding of this problem in OTR and may be of benefit in the design of a more rational clinical follow up of these patients.

In this concluding chapter, the descriptive as well as analytic epidemiological aspects of cancer and skin diseases in OTR are discussed in view of new evidence and recent findings by others. In addition, suggestions for future research are provided.

Descriptive epidemiology

Cancer

There is abundant evidence that the incidence of cancer is increased in OTR compared with the general population [1-9]. We confirmed the high burden of non-melanocytic skin cancer (NMSC), melanocytic skin cancer and non-cutaneous malignancies (NCM) in kidney transplant recipients (KTR) (**Chapter 2**) and simultaneous pancreas kidney transplant recipients (SPKTR) (**Chapter 5**). Cancers of the oral cavity, stomach, female genital organs, kidney, thyroid gland, but also leukemias and lymphomas, occurred 2 to 10 times more often compared with the general population. For SCC the standardized morbidity ratio even was as high as 40 (**Chapter 2)**. Many of the cancers that occurred at increased rates were those with a known or suspected infectious cause. Rates of stomach carcinomas were more than doubled and H. Pylori is estimated to cause over 60% of all stomach cancer [10]. Infection with human papillomavirus type 16 and 18 are causing cervical cancer [11, 12] and may also play a role in the etiology of a part of cancers of the oral cavity [13]. Lymphomas are related to Epstein - Barr virus infection [14, 15]. Several studies have suggested a possible causal role of beta- and maybe gamma-papillomavirus infections in the pathogenesis of cutaneous SCC, either directly, or in conjunction with sun exposure [16-21]. Since the immune system

may be crucial in protection from these infection related malignancies, these types op cancers may develop predominantly in immunocompromised OTR. Therefore, prevention and treatment of infections in OTR may reduce the incidence of malignancies.

We also confirmed the findings of previous studies [22-24] that there is a very high risk of subsequent NMSC after the first one (**Chapter 3**) and we found an increased risk of SCC in patients with a prior BCC and vice versa (**Chapter 6**). Furthermore, we have shown that OTR with SCC mainly developed new SCC and those with BCC mainly developed BCC (**Chapter 3**). Possibly, this could be explained by genetic predisposition or by different lifestyle factors of the patients, since the risk of SCC is considered to be associated with chronic sun exposure, whereas BCCs are more associated with intermittent, intense sun exposure [25]. Another explanation could be of immunologic nature. We hypothesize that a state of immune unresponsiveness may have been induced by the occurrence of the first skin cancer with immunologic tolerance to subsequent skin cancers with the same antigenic profile as the possible result.

We have also found an increased risk of NCM in OTR who developed SCC and BCC, but not the other way around (**Chapter 6**). The increased risks of NCM after the development of a prior SCC or BCC are in line with findings in the general population [26-28]. An inherited predisposition of cancer, a suboptimal immune response or lifestyle factors (smoking, sun exposure) are all possible explanations for the increased risk of NCM in patients with a prior SCC or BCC. Future research may provide insights in shared mechanisms of immunity against these types of cancer. We did not demonstrate an increased risk of SCC or BCC after the development of NCM, which is in contrast to findings in the general population [29]. Firstly, this difference may be explained by a lack of power due to the smaller population in our study compared with the 760 000 patients studied by Hemminki et al. Secondly, due to surveillance bias it is difficult to prove the association, since patients with NCM have a high probability of death soon after the NCM has been diagnosed, which has been shown in **Chapter 2**. A higher mortality rate is not observed in patients with SCC or BCC (**Chapter 2**). So far, cardiovascular diseases are the leading cause of death in OTR (30-50%), followed by infection (17-30%), but as a consequence of longer patient survival and older recipient age, malignancies have appeared as the third highest cause of mortality (8-18%) [30] and some authors believe that it will surpass cardiovascular diseases as the main cause of death in the coming years [31]. Therefore, it is very important that future research focuses on the prevention of malignancies.

Skin diseases

Compared with the large number of studies focusing on the development of skin cancer in OTR, infectious and inflammatory skin diseases have been studied less frequently [16, 19, 32-37]. Despite different methods however, all of these studies concluded that the prevalence of skin infections is very high with frequencies varying from 55-97% (Table 1) [33-37]. In **Chapter 4** we confirmed the high burden of skin diseases, and many patients developed multiple or recurrent skin diseases. The spectrum of skin diseases changed considerably with increasing time after transplantation and confirmed earlier publications that skin infections (e.g. herpes and candida) already occur early after transplantation [33, 34, 38], while most skin cancers increase exponentially with increasing time after transplantation [9, 39-42]. Although little is known about vascular skin problems after organ transplantation, there are some studies describing both arterial and venous vascular complications in KTR [43, 44]. In our cohort a significant proportion (8%) of the OTR does have some type of skin condition related to vascular diseases (**Chapter 4**). These data indicate that dermatologic care in OTR should not only be focused on skin malignancies, but also on skin infections and vascular skin diseases.

Analytic epidemiology

Immunosuppressive therapy

So far, there is no convincing epidemiological evidence for differences in oncogenic potential between the specific immunosuppressive agents. Comparison of incidence rate by type of immunosuppressive drug is difficult, because the regimen of immunosuppressive agents is strongly associated with the time period in which the patient is transplanted and the time period of transplantation has a profound effect on the risk of cancer. A recent study showed that treatment with azathioprine (Aza) was associated with a significant increased risk for SCC [45]. On the other hand, a randomized controlled trial in which patients were randomly allocated to one of three different treatment groups (Aza and prednisolone vs. long-term cyclosporine vs. short-term cyclosporine with a switch to Aza) from Australia, suggest that Aza and cyclosporine-based regimens are associated with similar overall long-term skin cancer risk after a follow up of 20 years, suggesting that the risk may be mediated by the total burden of immunosuppression rather than the agent [46]. Our studies provided additional evidence that Aza compared with other immunosuppressive drugs may increase the risk of both first and subsequent SCC (**Chapter 3 and 5**). Aza has been recognized to

Table 1 Skin diseases and (skin) cancer with increased risk in OTR based on the literature and this thesis.

Generally starting < 1 year after transplantation	Generally starting > 1 year after transplantation
Skin infections	**Skin infections**
Herpes	Human papilloma virus (warts)
Folliculitis	Erysipelas
Tinea versicolor	Dermatomycosis
Candidiasis	Onychomycosis
Skin inflammation	**Skin inflammation**
Acne	Dermatitis
Alopecia	
Skin miscellaneous	**Skin miscellaneous**
Oedema	Vascular problems
Hypertrichosis (Cyclosporin)	
Drug reactions	
Benign skin tumours	**Benign skin tumours**
Mollusca (poxvirus)	Warts (human papillomavirus)
	Seborrheic keratosis
	Cysts
	Lipoma
(Pre)malignant skin tumours	**(Pre)malignant skin tumours**
Kaposi sarcoma	Actinic keratoses
	Bowen's disease
	Keratoacanthoma
	Squamous cell carcinoma
	Basal cell carcinoma
	Malignant melanoma
	Merkel cell carcinoma
	Other adnex tumors
	Cutaneous lymphoma
Non skin cancer	**Non skin cancer**
Post-transplant Lymphoproliferative disorder	Lymphoma
	Leukemia
	Internal

increase photosensitivity of the skin and also enables UVA to directly damage DNA [47]. These characteristics of Aza may increase the risk of both first as well as subsequent SCC in patients who are chronically using this drug. Because most modern transplant regimens use combinations of mycofenolatemofetil, the calcineurin antagonist

tacrolimus and mTOR-inhibitors sirolimus and everolimus, future research should focus on these novel immunosuppressive agents. There is evidence to suggest that sirolimus compared with other immunosuppressive medications may confer a decreased risk of skin cancer [48, 49] due to its antiangiogenic effect, resulting in impaired tumor development. This is currently studied at our institute in both animal and human experimental studies (RESCUE trial).

Patients who are rejecting their grafts are treated with high doses of immunosuppressive therapy, including treatments with methylprednisolone, anti-thymocyte globulin (ATG) and muronomab-CD3 (OKT3) [50, 51]. One could speculate that these high doses of immunosuppressive rejection therapy lead to a state of severe immune deficiency and an increased risk of malignancies. However, there is only one study showing that rejection treatments are associated with a higher risk of SCC [52]. Using high serum creatinine levels at 1 year after transplantation as a measure of graft rejection, Bordea et al showed that patients with high serum creatinine levels had a higher risk of developing skin cancer [53]. They postulated that patients with a high serum creatinine level had maintained higher levels of immunosuppression to prevent rejection, which may have led to a higher risk of skin cancer [53]. However, Bordea et al, did not observe an increased incidence of skin cancer in patients receiving additional immunosuppression in the form of rejection treatments with ATG and OKT3, which is in line with several other studies [53-56]. We provided additional evidence that rejection therapy is not associated with an increased risk of malignancies. First, in **Chapter 5** we have shown that impending graft rejection, and the subsequent rejection therapies were not associated with SCC or BCC. In addition, **Chapter 7** showed that an increased number of transplantations are associated with a decreased risk of both SCC and NCM. Patients with multiple transplantations have rejected previous grafts, and are therefore treated with high levels of immunosuppression, including OKT3 and/or ATG to prevent final rejection, during short periods.

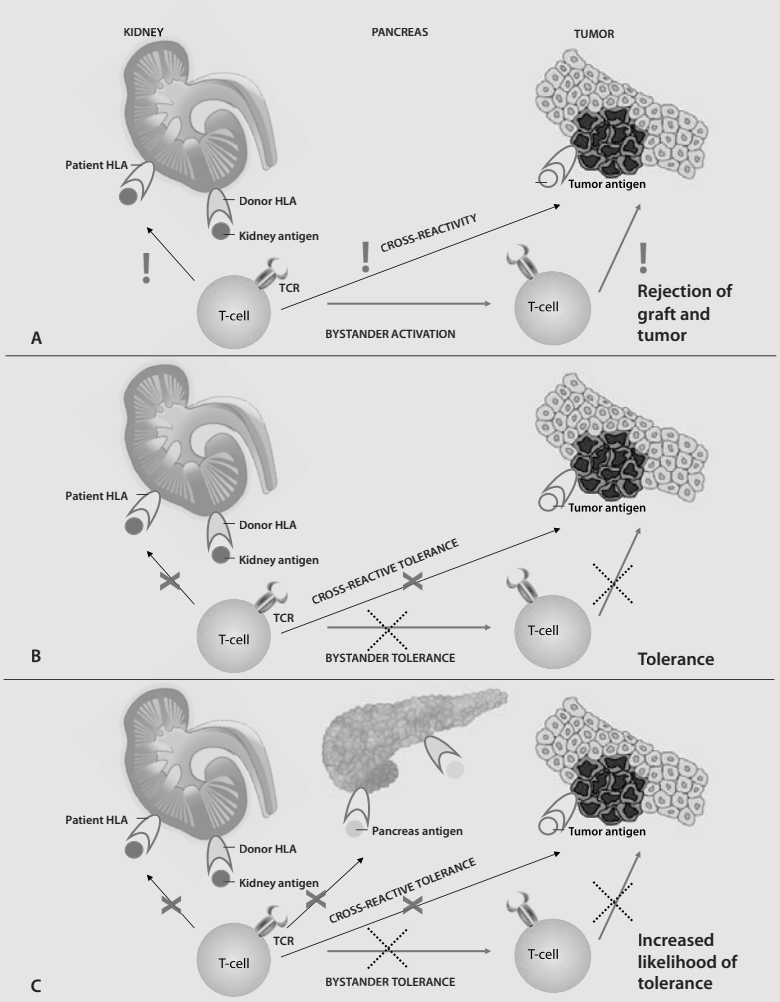
From 1991 until 1993, SPKTR are routinely treated with OKT3 as induction therapy and since 1998, SPKTR are routinely treated with high dose of induction therapy with ATG or daclizumab or in exceptional cases with basiliximab to prevent later rejection. In **Chapter 5** we have shown that induction treatments, similarly as rejection treatments, are also not associated with cancer. Apparently, the high levels of immunosuppression in induction and rejection treatments with the expected lower activated immune response during these periods do not lead to an increased risk of malignancies.

Shared immunity against organs and tumors

In **Chapter 7** we have shown that the rejection rate is associated with a decreased risk of cancer. Patients with three and more transplantations had a 1.6 respectively 3.6-times decreased risk of both NCM and SCC. A mechanism which may explain the prevention of malignancies in OTR is heterologous immunity, which is partial immunity that can occur in response to an antigen if the host has been previously immunized with an unrelated antigen [57]. This can be due to bystander activation or cross-reactivity, which is a reaction of a T-cell against more than one antigen. For example, some human virus-specific T-cells have been shown to recognize antigens in other HLA molecules [58]. We speculate that in patients with multiple transplantations bystander activation or cross-reactivity between donor grafts and malignancy-associated antigens result in protection from development of malignancies (Figure 1 A).

Supporting the hypothesis of cross-reactivity between donor graft and malignancy, we have found evidence that the opposite mechanism may also occur by induction of cross-reactive tolerance. Besides immunosuppressive therapy and other risk factors induction of immunologic tolerance may play a role in the development of malignancies in OTR (**Chapter 5 en 7**). Immunologic tolerance is a state of immune unresponsiveness to specific antigens induced by previous exposure to these antigens. Tolerance may result from T cells recognizing their antigen in a tolerizing environment, such as in the presence of immunosuppressive drugs, which may cause suppression, functional inactivity or apoptosis of the T cells [59]. Cross-reactive tolerance against antigens derived from donor organs has been demonstrated in animal models. Rats who received a heart in combination with a lung or spleen were more tolerant for the transplanted heart, since a reduced rejection rate of the transplanted heart was observed [60]. We have shown that SPKTR have an increased risk of SCC compared with KTR (**Chapter 5**). We speculate that the transplanted pancreas may have induced more tolerance against donor antigens presented in patient or donor HLA molecules. An increased cross-reactive tolerance against SCC-associated antigens in the host could then lead to an increased risk of SCC in SPKTRs (Figure 1 B and C). This could potentially affect SCC more severely than BCC, as SCCs are more antigenic cancers than are BCCs [61]. Similarly, tolerance may develop towards antigenic NCM. Induction of tolerance is a major goal in graft transplantation but these data suggest that the induction of tolerance could lead to unwanted side effects, such as an increased risk of infections and malignancies. Animal studies which should point out whether this hypothesis is true should be performed, so that we can learn more about the underlying mechanism and the role of different immunosuppressive agents, modulating this process.

Figure 1 Mechanism of shared immunity against organs and tumors.



A. The T cell is activated after recognition of its specific kidney antigen in a patient or donor HLA molecule, and after recognition of its specific tumor antigen in a patient HLA molecule due to cross-reactivity, which may result in rejection of both kidney and tumor. Alternatively, another T cell recognizing the tumor antigen is activated due to bystander activation, which may result in rejection of the tumor. **B**. The T cell does not respond to recognition of its specific kidney antigen in a patient or donor HLA molecule, and neither to its specific tumor antigen in a patient HLA molecule due to cross-reactive tolerance. Alternatively, another T cell recognizing the tumor antigen does not respond due to bystander tolerance. **C**. Similar to B., but the presence of an increased number of donor antigens derived from the additional transplanted organ increases the likelihood of occurrence of cross-reactive tolerance or bystander tolerance.

TCR: T cell receptor, HLA: human leukocyte antigen

Guidelines for dermatologists

In Table 2 we summarize clinical predictors for SCC in OTR to distinguish high risk and low risk patients for the development of SCC. The risk factors hatched in grey were studied in this thesis. The others are known risk factors from literature [18, 39, 53, 62, 63]. To reduce the tumor burden in OTR, the management of these patients requires an interdisciplinary approach including education about photoprotection, revision of immunosuppression and adequate dermatological treatments. Prevention of skin cancer in OTR will depend on better patient education. Awareness about skin cancer risk, and compliance with photoprotective measures, have indeed improved with proper dissemination of information to OTR in specialized dermatology clinics [64]. Therefore, we recommend that all candidates for transplantation should receive oral and written information about dermatological complications after transplantation and advice on sun-protective clothing and the use of sunscreen to avoid these complications. There is a high need for developing guidelines of dermatological care for OTR as these patients represent a significant and increasing challenge to dermatologists. In Table 3 we provide a schematic proposal for dermatological evaluation and aftercare in OTR based on the results of this thesis and the literature [65]. Early diagnosis through regular and appropriate follow up preferably in specialized dermatology clinics for OTR is strongly recommended. We therefore advise to check each OTR at least every two years. Since time after transplantation, resulting in more years on maintenance immunosuppression, and older age were risk factors for SCC (**Chapter 2-7**) we advise to check patients using immunosuppression for more than 10 years or being older than 50 years of age once a year. In these non sun damaged patients the focus should be on sun protective measures. Independent on age and time on immunosuppression, when patients have signs of severe or moderate sun damage, we recommend to checking them twice a year. Individual primary actinic keratoses (AK) can be treated with cryosurgery or topical application of imiquimod or 5-fluorouracil. However, this does not prevent the occurrence of new AK, since the areas of 'field cancerisation', where a discrete area of tissue is at increased risk of developing skin cancer, are not cured. Systemic retinoids can be used for chemo-prevention since there are studies suggesting that these drugs reduce the number of preexisting AK and slow down the development of new lesions [66-68]. Tolerability of the drug, however, is a major factor limiting its use [67]. In addition, it seems reasonable to consider revision of immunosuppression in patients with AK, both by reduction of the immunosuppressive dose [22] or conversion to sirolimus [48, 49], although more studies are needed to determine the potentially beneficial effect of these measures. Since we

Table 2 Risk factors for squamous cell carcinoma in organ transplant recipients. Risk factors studied in this thesis are hatched in grey.

Risk factors	No apparent risk factor	Protective factors
Chronic sun exposure	Donor type (living/ cadaver)	Multiple kidney transplantations
Painful sunburns	Rejection therapy	Sirolimus versus other maintenance immunosuppression
Fitzpatrick skin type I and II	Induction therapy	Fitzpatrick skin type V and VI
High number of keratotic skin lesions (risk indicator)	HLA mismatching	
Human papillomavirus infection		
Smoking		
Male		
Older age (at transplantation)		
Azathioprine versus other maintenance immunosuppression		
Simultaneous pancreas kidney transplantation		
Previous diagnosis of SCC/BCC/NCM		
Longer time since transplantation / years on immunosuppression		

have shown that the first SCC serves as a predictive marker for multifocal tumor development (**Chapter 3**), patients with a previous SCC, but also patients with previous BCC or NCM, should be considered as a high risk group and should therefore be checked at least 4 times a year (Table 3). All OTR with rapidly growing (and often

painful) lesions should be seen within 1 to 2 weeks. In OTR with suspected or biopsy-proven SCC, surgery with histology-controlled margins is the gold-standard therapy. In patients with multiple SCC, curettage and electrodessication can be used for lesions on the trunk and extremities, since it has been shown that this is an effective treatment for SCC in OTR [69, 70] with a low recurrence rate [69]. However, strict follow-up in specialized dermatology clinics in OTR with multiple SCC is necessary.

Summary and concluding remarks

Descriptive epidemiologic data in this thesis demonstrated and confirmed the major morbidity of NCM, NMSC and other skin diseases in OTR. Analytic epidemiologic data in this thesis showed that Aza as maintenance immunosuppressive drug is a risk factor for first and subsequent SCC. Furthermore, SPKTR have a highly increased risk to develop SCC compared with KTR and the rejection rate was shown to be associated with a decreased risk of cancer. To our knowledge, these are the first data suggesting that besides immunosuppressive therapy, induction of immunologic tolerance may play a role in the development of malignancies.

Since many malignancies and skin diseases were related to an infectious cause, future studies should point out whether prevention and treatment of infections will reduce the incidence of both (skin) malignancies and (skin) infections. Considering the harmful effects of the classical immunosuppressive agent Aza and the promising anticarcinogenic effects of mTOR inhibitors, future studies should aim to study the effect of this novel drug class on the risk of malignancies. Since induction of tolerance, which is a major goal in graft transplantation, could possibly result in unwanted side effects, such as an increased risk of infections and malignancies, it is very important that future animal studies should be performed to learn more about the underlying mechanisms.

As far as the mechanisms of tolerance are not clarified yet, the frequent occurrence of malignancies in OTR due to immunosuppression should be managed adequately by well educated physicians in near future. A proper guideline is needed to provide optimal management of OTR, to prevent and reduce morbidity and mortality due to infections and malignancies in these patients.

Table 3 Schematic proposal for dermatological evaluation and aftercare in organ-transplant recipients.

Number of recommended visits to dermatology outpatient clinic	Patient characteristics	Management
Once in two years	No keratotic skin lesions/ no sun damage No history of cutaneous malignancies Fitzpatrick skin type V-VI Less than 10 years after transplantation < 50 years of age	Sun protective measures
Once a year	No keratotic skin lesions/ no sun damage No history of cutaneous malignancies More than 10 years after transplantation OR > 50 years of age	Sun protective measures
Two times a year	Low number (less then 10) keratotic skin lesions/ Severe to moderate sun damage No history of cutaneous malignancies	Sun protective measures Consider systemic retinoids Revision of immunosuppression Cryosurgery/topical imiquimod/ 5-fluorouracil
Four times a year	Patients with more then 10 keratotic skin lesions/severe sun damage Patients with a history of one SCC/BCC	See above plus Complete surgical removal
Five and more times a year	Multiple previous SCC/BCC	See above plus Complete surgical removal Consider curettage and coagulation

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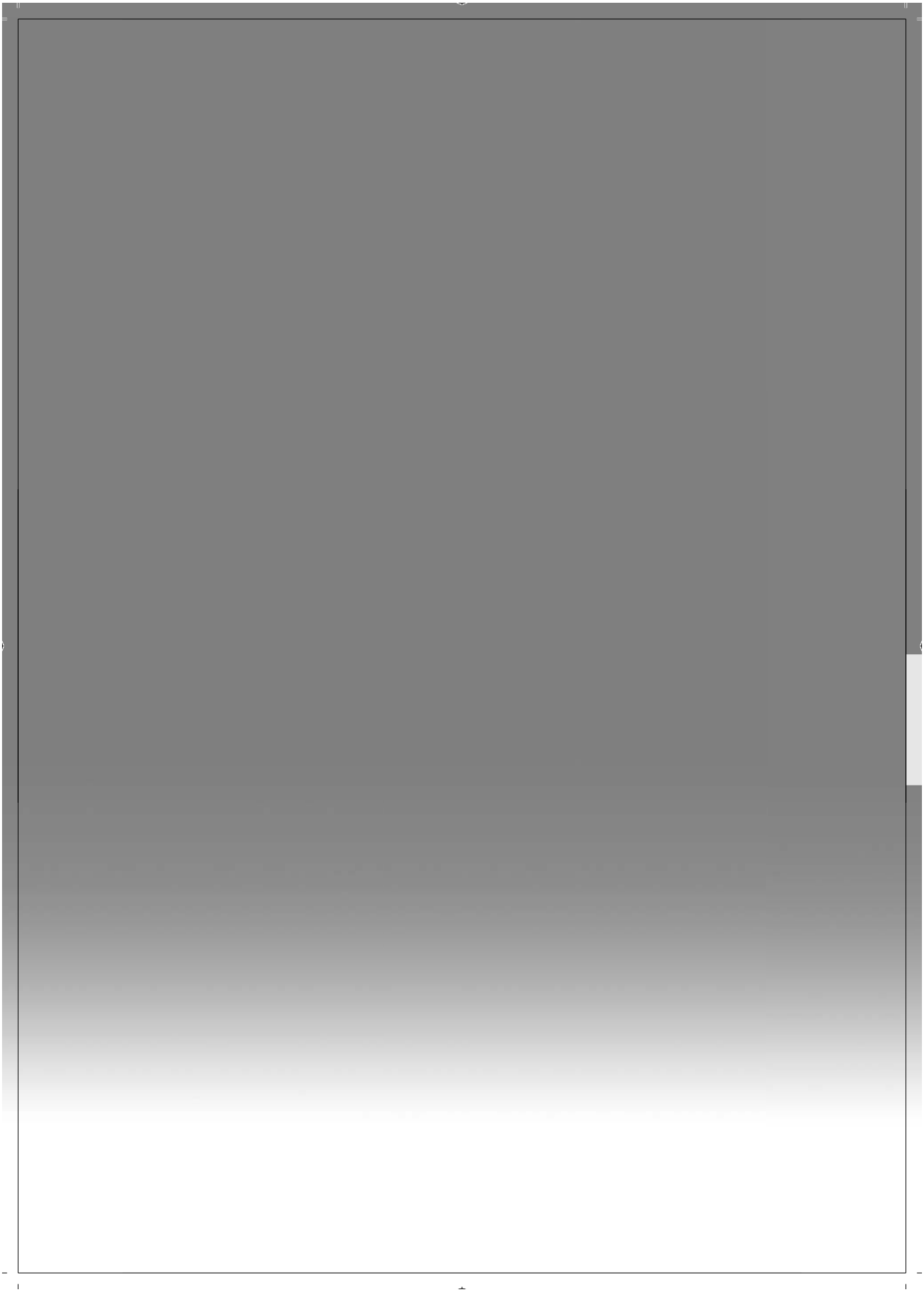

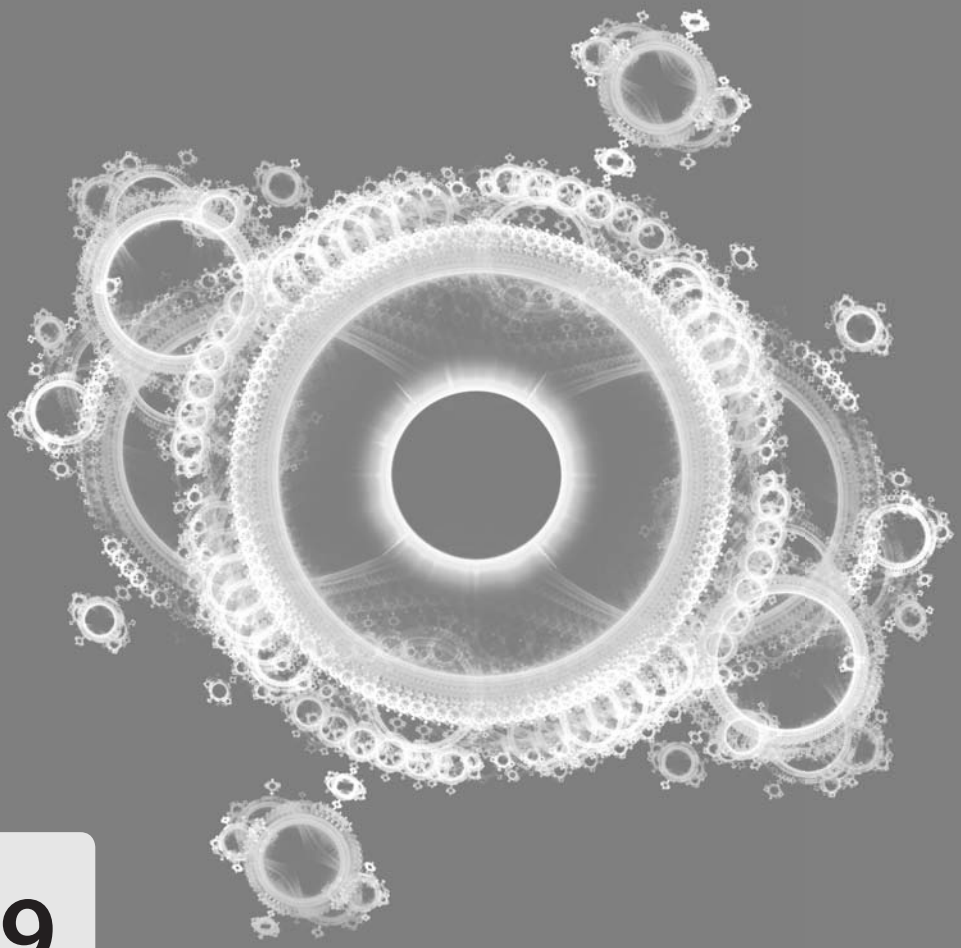

9

Nederlandse samenvatting
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Nawoord

Nederlandse samenvatting

De eerste succesvolle orgaantransplantie werd in Boston in 1954 uitgevoerd en betrof een transplantatie van een nier tussen twee individuen die genetisch identiek zijn, een eeneiige tweeling. Snel daarna werd het door de komst van geneesmiddelen die het afweersysteem onderdrukken (immuunsuppressieve geneesmiddelen) ook mogelijk organen te transplanteren tussen individuen die genetisch niet identiek zijn. Vanaf 1966 werden niertransplantaties uitgevoerd in het Leids Universitair Medisch Centrum (LUMC). Inmiddels is het ondergaan van een niertransplantatie de beste behandeling voor patiënten met ernstig nierfalen. Ondanks het succes van deze therapie zijn er ook complicaties. Vanwege het chronisch gebruik van immuunsuppressieve geneesmiddelen hebben orgaantransplantatiepatiënten een verhoogd risico op het krijgen van kanker en infecties. Vooral het risico op niet-gepigmenteerde huidkankers, in het bijzonder het plaveiselcelcarcinoom, is sterk verhoogd in deze groep patiënten vergeleken met de algemene populatie. Over de exacte incidentie van de verschillende typen kanker en (huid)infecties evenals risicofactoren die de kans op deze aandoeningen verhogen bestaat nog onduidelijkheid. In dit proefschrift hebben wij getracht meer inzicht te verschaffen in de epidemiologische aspecten van kanker en (huid)infecties in orgaantransplantatiepatiënten. In het eerste deel (**hoofdstuk 2-4 en 6**) wordt de frequentie van ziekten onderzocht (beschrijvende epidemiologie), terwijl in het tweede deel (**hoofdstuk 5 en 7**) risicofactoren (analytische epidemiologie) voor het ontstaan van deze aandoeningen worden bestudeerd.

Hoofdstuk 1 geeft een overzicht van het klinische probleem van (huid)kanker bij orgaantransplantatiepatiënten. De belangrijkste risicofactoren worden besproken, zoals immuunsuppressieve geneesmiddelen en ultraviolet licht. Daarnaast wordt beschreven wat er bekend is over andere huidaandoeningen dan huidkanker, bijvoorbeeld infecties van de huid, in deze patiëntengroep.

Risico op (huid)kanker

In **hoofdstuk 2** hebben we onderzocht wat de incidentie is van kanker in patiënten die een niertransplantatie hebben ondergaan tussen 1966 en 2006 in het LUMC. Deze incidentie hebben we vergeleken met de incidentie in de algehele bevolking. Dertig jaar na transplantatie heeft 50% van de patiënten tenminste een vorm van kanker, waarbij het plaveiselcelcarcinoom van de huid de meest frequent voorkomende kanker is. Kanker van de keel, slokdarm, maag, nier, schildklier, lymfeklier, beenmerg

en vrouwelijk geslachtsorgaan komt 2-10 keer vaker voor bij niertransplantatiepatiënten dan bij de algehele bevolking. Het plaveiselcelcarcinoom van de huid komt zelfs 40 keer vaker voor. Bij veel van deze typen kanker bestaat een sterke relatie met infecties. Omdat het afweersysteem belangrijk is bij de protectie tegen deze infecties, lijkt het verklaarbaar dat juist deze typen van kanker veel voorkomen bij transplantatiepatiënten. Veel van de resultaten van deze studie komen overeen met eerder beschreven studies. Het verhoogde risico op het ontstaan van schildklierkanker was slechts een keer eerder beschreven. Mogelijk speelt ook hier een infectie een rol.
Hoewel het risico op een eerste huidkanker in patiënten met een orgaantransplantatie goed bekend is, is er weinig bekend of transplantatiepatiënten meer dan een huidkanker ontwikkelen en hoe snel deze ontstaan nadat de eerste huidkanker zich heeft ontwikkeld. In **Hoofdstuk 3** hebben we dit apart onderzocht voor twee typen huidkanker, het plaveiselcelcarcinoom en het basaalcelcarcinoom. Van de patiënten met een niertransplantatie die een plaveiselcelcarcinoom ontwikkelden kreeg 75% binnen 5 jaar minimaal een extra plaveiselcelcarcinoom. Dit percentage is veel hoger dan bij de algemene populatie waar slechts 20% van de patiënten met een plaveiselcelcarcinoom een tweede tumor heeft ontwikkeld na 5 jaar. Van de patiënten met een basaalcelcarcinoom kreeg 51% tenminste een tweede tumor na 5 jaar, wat wel vergelijkbaar is met het percentage dat wordt gezien bij de algemene populatie.

Risico op andere huidziekten dan huidkanker

In tegenstelling tot het ontstaan van huidkanker bij orgaantransplantatiepatiënten is er weinig bekend over andere huidaandoeningen die worden gezien bij orgaantransplantatiepatiënten. In **hoofdstuk 4** wordt een overzicht gegeven hoeveel patiënten die een nier of een nier en alvleesklier getransplanteerd hebben gekregen tussen 1966 en 2006 in het LUMC in datzelfde ziekenhuis zijn gezien op de afdeling dermatologie tussen 1994 en 2006. In totaal werden er 2408 huidziekten geregistreerd in 801 van de 1768 orgaantransplantatiepatiënten. De meest frequent voorkomende diagnose was huidinfectie (24%), gevold door benigne huidtumor (23%) en maligne huidtumor (18%), waaronder het plaveiselcelcarcinoom en het basaalcelcarcinoom valt. Opvallend is dat het spectrum van huidziekten verandert gedurende de tijd na transplantatie. De eerste jaren na transplantatie domineren huidinfecties, zoals huidafwijkingen ten gevolge van het herpes simplex virus of de schimmel Candida Albicans, terwijl langer na transplantatie huidtumoren domineren. Omdat er ook patiënten in andere ziekenhuizen door dermatologen zijn gezien of een huisarts

hebben geconsulteerd zal het aantal patiënten met huidafwijkingen na het ondergaan van een orgaantransplantatie waarschijnlijk nog hoger liggen en beschrijft deze studie waarschijnlijk een onderrapportage van de werkelijke frequentie van huidziekten in deze groep patiënten.

Risicofactoren voor huidkanker

Immuunsuppressieve geneesmiddelen

Een belangrijke risicofactor in de ontwikkeling van huidkanker bij orgaantransplantatiepatiënten is het gebruik van immuunsuppressieve geneesmiddelen die deze patiënten gebruiken om afstoting van het orgaan te voorkomen. Het afweersysteem is door langdurig gebruik van deze medicijnen niet meer in staat om kankercellen op te ruimen. Er zijn veel verschillende typen immuunsuppressieve geneesmiddelen in orgaantransplantatiepatiënten. Sommige geneesmiddelen, zoals azathioprine, hebben niet alleen een immuunsuppressief effect, maar ook een direct effect op huidcellen dat tot kanker zou kunnen leiden. In tegenstelling tot dit klassiekere immuunsuppressieve geneesmiddel hebben de nieuwere middelen, zoals sirolimus, mogelijk juist een remmende werking op de tumorgroei. In **hoofdstuk 3 en 5** worden verschillende immuunsuppressieve geneesmiddelen als risicofactor op het krijgen van eerste en volgende plaveiselcelcarcinomen van de huid onderzocht. Beide studies laten zien dat het geneesmiddel azathioprine vergeleken met andere immuunsuppressieve geneesmiddelen een hoger risico geeft op het ontwikkelen van huidkanker. Het gegeven dat azathioprine de fotosensitiviteit van de huid verhoogt en in staat is om in combinatie met ultraviolet A licht directe schade aan het DNA kan geven, is mogelijk een verklaring voor het verhoogde risico.

Naast de bovengenoemde immuunsuppressieve geneesmiddelen, die orgaantransplantatiepatiënten chronisch moeten gebruiken (onderhoudsgeneesmiddelen), is het soms nodig om tijdelijk aanvullende geneesmiddelen voor te schrijven, bijvoorbeeld als er aanwijzingen zijn dat het orgaan wordt afgestoten ondanks de onderhoudsgeneesmiddelen. Dit noemen we rejectiebehandelingen en betreft kortdurende maar hoge doseringen immuunsuppressieve behandelingen met methylprednisolon, anti-thymocytglobuline en muronomab. In **hoofdstuk 5** hebben wij aangetoond dat het geven van rejectiebehandelingen geen extra risico vormt voor het ontwikkelen van een plaveiselcelcarcinoom van de huid. Mogelijk komt dat omdat deze middelen slechts kortdurend gebruikt worden en speelt het chronisch gebruik van immuunsuppressieve geneesmiddelen een belangrijkere rol.

De aanwezigheid van kanker

In **Hoofdstuk 6** hebben we onderzocht of orgaantransplantatiepatiënten die kanker van de interne organen ontwikkelden een hoger risico hebben op het ontwikkelen van huidkanker. Op basis van de resultaten van onze studie blijkt dit niet het geval te zijn, terwijl in eerdere studies onder de algehele bevolking wel een hoger risico werd gevonden. Mogelijk is de mortaliteit van orgaantransplantatiepatiënten met kanker zo hoog dat zij niet meer de kans krijgen om een huidtumor te ontwikkelen. Andersom wordt er in deze studie wel een verhoogd risico gevonden voor het ontwikkelen van kanker bij orgaantransplantatiepatiënten met huidkanker. Patiënten met een plaveiselcelcarcinoom van de huid hebben een 3.5 keer verhoogd risico op het ontwikkelen van kanker van de interne organen en patiënten met een basaalcelcarcinoom hebben een 2.1 keer verhoogd risico op het krijgen van kanker van de interne organen. Genetische factoren, bepaalde leefstijlgewoonten of immuunfactoren kunnen deze bevinding mogelijk verklaren.

Aantal en type transplantaties

Hoofdstuk 7 beschrijft een studie waarin onderzocht is of patiënten die vaker een orgaantransplantatie ondergingen een ander risico op kanker hebben dan patiënten die slechts een keer een orgaan getransplanteerd kregen. De resultaten laten zien dat patiënten die 3 keer of vaker een orgaan getransplanteerd kregen een 1.6 keer verlaagd risico hebben op kanker. Voor het plaveiselcelcarcinoom van de huid is dit risico zelfs 3.6 keer lager. Het is beschreven dat bepaalde immuuncellen (T-cellen) een afweerreactie genereren tegen meer dan een antigeen. Dit wordt crossreactieve immuunrespons genoemd. Wij speculeren dat patiënten die vaker een orgaantransplantatie hebben ondergaan niet alleen een afweerreactie hebben tegen het getransplanteerde orgaan met afstoting tot gevolg, maar ook een afweerreactie tegen kanker geassocieerde antigenen. Hoewel het exacte mechanisme nog niet duidelijk is wordt onze hypothese bijgestaan door resultaten beschreven in **hoofdstuk 5**. Als een patiënt een orgaan getransplanteerd krijgt en dit niet als lichaamsvreemd wordt gezien spreekt men van tolerantie. Zoals T-cellen een crossreactieve immuunrespons tegen meerdere antigenen kunnen vertonen, kan ook crossreactieve tolerantie tegen meerdere antigenen optreden. Een voorbeeld van de ontwikkeling van tolerantie is de betere functie en overleving van een transplantaat in patiënten die voorafgaand aan de transplantatie een bloedtransfusie hebben ontvangen. Daarnaast is aangetoond dat er minder afstoting was in ratten die naast een hart ook een long of milt getransplanteerd kregen. In **hoofdstuk 5** hebben wij aangetoond dat patiënten die zowel een nier als een alvleesklier getransplanteerd kregen een

6 keer hoger risico hadden op het plaveiselcelcarcinoom dan patiënten die alleen een nier getransplanteerd kregen. Na correctie voor immuunsuppressieve geneesmiddelen was dat risico nog steeds 3 keer verhoogd. Deze bevinding doet veronderstellen dat het verhoogde risico op kanker bij transplantatiepatiënten met een nier en alvleesklier mogelijk ten dele toe te schrijven valt aan het ontwikkelen van meer tolerantie door de aanwezigheid van twee organen.

Hoofdstuk 8 geeft een samenvatting van de resultaten beschreven in de voorgaande hoofdstukken en vervolgens worden de bevindingen bediscussieerd. Op basis van onze bevindingen beschreven in dit proefschrift worden adviezen gegeven om de zorg van orgaantransplantatiepatiënten te verbeteren in een praktische richtlijn voor dermatologen. Omdat veel van de typen (huid)kanker en huidziekten bij transplantatie-patiënten een relatie hebben met infecties, zou toekomstig onderzoek zich kunnen richten op de mogelijkheden van behandeling en preventie van deze infecties met als doel om de incidentie van deze latere aandoeningen te reduceren. Gezien de veelbelovende anticarcinogene effecten van sirolimus zouden toekomstige studies erop gericht moeten zijn het effect van deze nieuwe immuunsuppressieve geneesmiddelen op het risico van kanker te onderzoeken. Omdat het induceren van tolerantie bij orgaantransplantatie een belangrijk doel is, maar mogelijk ongewenste bijwerkingen geeft zoals een toegenomen risico op infecties en kanker, is het van belang om het mechanisme van tolerantie beter te begrijpen.

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List of abbreviations

AK	Actinic keratoses
ATG	Anti-thymocyte globulin
Aza	Azathioprine
BCC	Basal-cell carcinoma
CsA	Cyclosporine A
DBC	Diagnosis treatment combination
HLA	Human leucocyte antigen
HPV	Human papillomavirus
HTR	Heart transplant recipients
KTR	Kidney transplant recipients
LUMC	Leiden University Medical Center
MEDREG	Medical registration program
MMF	Mycofenolatemofetil
NCM	Non-cutaneous malignancy
NMR	National Medical Registration
NMSC	Non-melanocytic skin cancer
OKT3	Muromonab
OTR	Organ transplant recipient
P	Prednisolone
SCC	Squamous-cell carcinoma
SIR	Standardized incidence ratio
SMR	Standardized morbidity ratio
SPKTR	Simultaneous pancreas kidney transplant recipients
Tac	Tacrolimus

Curriculum Vitae

Irma Wisgerhof is geboren op 28 mei 1981 te Papendrecht. Na het behalen van het VWO diploma aan de christelijke scholengemeenschap de Lage Waard te Papendrecht in 1999, begon zij in datzelfde jaar met de studie Farmacie aan de Universiteit Utrecht. Na het behalen van het propedeuse startte zij een jaar later aan dezelfde universiteit met de studie Geneeskunde. In deze periode verrichtte zij onderzoek naar het voorschrijfgedrag van antibiotica bij bovenste luchtweginfecties door huisartsen (dr. M. Kuyvenhoven). Zij liep haar co-schap Gynaecologie in Paramaribo (Suriname) en haar co-schap Dermatologie in Melbourne (Australië). Na een wetenschappelijke stage bij de afdeling Dermatologie van het Leids Universitair Medisch Centrum werd zij na het behalen van haar artsexamen in december 2006 aangesteld als AGIKO (assistent-geneeskundige in opleiding tot klinisch onderzoeker) op dezelfde afdeling. Dit leidde tot het in dit proefschrift beschreven onderzoek onder begeleiding van dr. J.N. Bouwes Bavinck en Prof. Dr. R. Willemze. In december 2009 is zij gestart met de opleiding tot dermatoloog (opleider Prof. Dr. R. Willemze).

Irma woont samen met Maarten Zandvliet en hun dochter Hannah.

Nawoord

Dit proefschrift is tot stand gekomen dankzij de medewerking van vele mensen van de afdelingen Dermatologie, Nefrologie, Immunohematologie en Medische statistiek. Een aantal mensen wil ik hiervoor in het bijzonder bedanken. Allereerst mijn co-promotor Jan Nico Bouwes Bavinck en promotor Rein Willemze dank dat ik de kans heb gekregen om dit onderzoek uit te voeren. Ron Wolterbeek, jouw onverminderd enthousiasme voor de statistiek is prijzenswaardig. Onze vele gesprekken hierover zijn de kwaliteit van diverse hoofdstukken zeker ten goede gekomen. Geert Haasnoot, Lydia van der Geest en Marco Mallat, ook jullie hebben een belangrijke bijdrage geleverd door het genereren van onmisbare data. Jeroen Edelbroek, bedankt voor het coderen en analyseren van data. Paul Douw van der Krap, jouw hulp bij de lay-out van vele figuren heb ik erg gewaardeerd. Daarnaast wil ik alle co-auteurs danken voor hun bijdrage aan het tot stand komen van de hoofdstukken. Dankzij jullie nuttige inbreng uit vaak heel verschillende invalshoeken hebben jullie mij vele malen aangezet tot kritisch nadenken. Ik heb veel van jullie geleerd.

Mijn collega's van het lab en van de polikliniek wil ik danken voor zowel de interesse in dit onderzoek als de afleiding van dit onderzoek. Zonder jullie waren de afgelopen jaren een stuk moeilijker en in ieder geval erg veel saaier geweest! Mede AGIKO's; regelmatig leidden onze discussies tot verscherpt inzicht, maar vooral tot hernieuwde energie, bedankt hiervoor.

Speciale dank aan mijn familie en vrienden voor hun voortdurende steun en in het bijzonder aan mijn ouders voor de liefdevolle basis die ze me hebben gegeven. Maarten, jouw enthousiasme voor de wetenschap vind ik bewonderenswaardig. Zowel aan jouw motiverende als relativerende eigenschappen heb ik de afgelopen onderzoeksjaren veel gehad. Samen met Hannah maak je me erg gelukkig.