ORIGINAL ARTICLE

Kidney Transplant Recipients with Cutaneous Squamous Cell Carcinoma Have an Increased Risk of Internal Malignancy

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This study aimed to investigate whether the occurrence of cutaneous squamous cell carcinomas (SCCs) is associated with an increased risk of internal malignancies (IMs) in kidney transplant recipients (KTRs). In a cohort study, all patients receiving kidney transplantation in Leiden, the Netherlands, between 1966 and 2006 were followed up. All malignancies that had developed between 1966 and 2007 were recorded. Time-dependent Cox regression analyses were used to calculate the association between the development of cutaneous SCCs and IMs. The incidence of IMs in the KTRs after transplantation was also compared with the general Dutch population by calculating standardized morbidity ratios (SMRs) and was matched for age, sex, and time period in which the malignancy had occurred. Among 1,800 KTRs, 176 (9.8%) developed cutaneous SCCs and 142 (7.9%) developed IMs after transplantation. In patients with prior cutaneous SCCs, the adjusted risk to develop IMs was 3.0 (1.9; 4.7). In KTRs without cutaneous SCCs, the risk of IM compared with the general population was hardly increased. KTRs with cutaneous SCCs have an increased risk to develop IMs, and this information can be used to identify KTRs who are at an increased risk for IMs.

INTRODUCTION

Kidney transplant recipients (KTRs), as well as other organ transplant recipients, have a 2- to 6-fold increased risk of malignancy compared with the general population (Hartevelt et al., 1990; London et al., 1995; Adami et al., 2003; Euvrard et al., 2003; Vajdic et al., 2006; Grulich et al., 2007; Villeneuve et al., 2007; Alam et al., 2011; Wisgerhof et al., 2011) and many KTRs develop multiple malignancies (Euvrard et al., 2006; Wisgerhof et al., 2010). In the Netherlands, the standardized morbidity ratio (SMR) for internal malignancies (IMs) in KTRs compared with the age- and sex-matched general population was 1.6 (95% confidence interval, 1.4; 1.9), and for cutaneous squamous cell carcinoma (SCC) the SMR was 39.6 (34.2; 45.9; Wisgerhof et al., 2011).

The risk of IMs after the occurrence of cutaneous SCCs has been studied in the general population. Some studies showed that patients with a cutaneous SCCs have a 2-fold increased risk of IMs (Levi et al., 1997; Karagas et al., 1998; Wassberg et al., 1999; Hjalgrim et al., 2000; Hu et al., 2005; Chen et al., 2008), but other studies showed no increased risk of IMs, or even a slightly decreased risk, after the occurrence of cutaneous SCCs (Levi et al., 1997; de Vries et al., 2007; Grant, 2007; Tuohimaa et al., 2007; Soerjomataram et al., 2008; Cantwell et al., 2009). As far as we know, no previous studies have investigated the association between cutaneous SCCs and IMs in KTRs.

The aims of this study were to investigate the risk of IMs after the occurrence of cutaneous SCCs in KTRs and to determine whether KTRs who develop cutaneous SCCs can be used to identify patients who are at an increased risk for IMs.

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Abbreviations: IMs, internal malignancies; KTRs, kidney transplant recipients; LUMC, Leiden University Medical Center; MMF, mycophenolate mofetil; SCC, squamous cell carcinoma; SMR, standardized morbidity ratio

RESULTS

Baseline characteristics of the KTRs

Between March 1966 and January 2006, 1906 patients received their first kidney transplant in Leiden (Wisgerhof et al., 2011). Figure 1 shows that 106 patients were excluded because of different reasons. Of the remaining 1,800 patients, 176 (9.8%) had developed one or more cutaneous SCCs and 142 (7.9%) had developed one or more IMs, whereas 1,521 (84.5%) KTRs did not develop any type of cancer. The baseline characteristics of the patients without and with cancer are provided in Table 1. This cohort may differ from
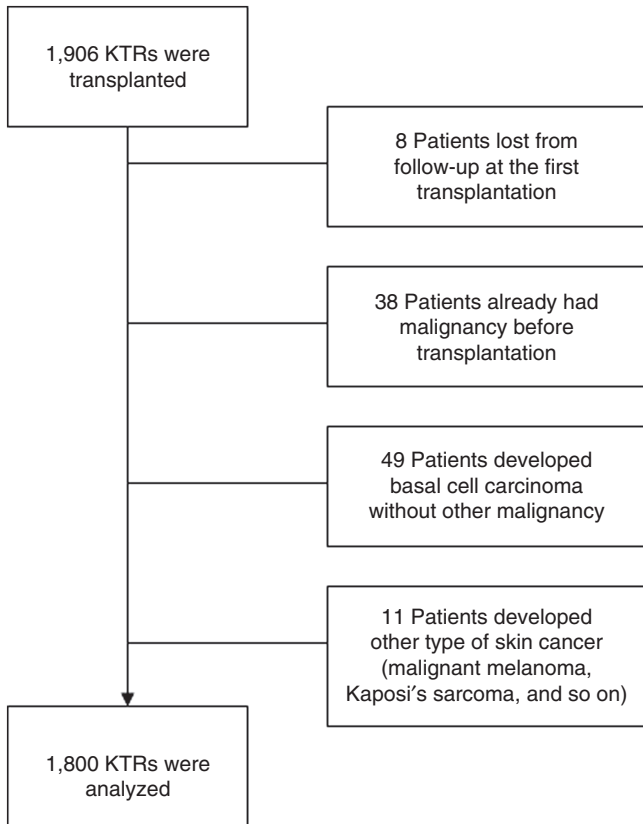
Figure 1. **Selection of kidney transplant recipients (KTRs) for this study.**

other centers, because of the large number of KTRs who were transplanted before 1986 and the high number of KTRs on maintenance therapy with azathioprine (Aza) in any combination. The median age at transplantation of the 1,800 KTRs together was 43 years (ranging from 4 to 77 years), with a median follow-up of 11 years (ranging from 0.1 to 40 years); 62% were male patients, which is caused by a predominance of kidney problems in the male population.

Prognostic factors for cancer
Non-adjusted and adjusted hazard ratios to develop cutaneous SCC or IM are presented in Table 2. Increased age at transplantation was, as could be expected, a statistically significant prognostic factor for both cutaneous SCCs and IMs. Male sex was a significant risk factor for cutaneous SCCs but not for IMs. KTRs immunosuppressed with Aza had the highest risk to develop both cutaneous SCCs and IMs, but for this association there was some confounding by the time period of transplantation. This is illustrated by the hazard ratio of 1.7 for developing IMs in KTRs who were immunosuppressed with cyclosporine A or tacrolimus compared with Aza, which reverted to 0.83 after adjustment for time period of transplantation (Table 2).

The time period of transplantation had an important impact on the risk of cutaneous SCCs but not on the risk of IMs. After 1986, the risk of cutaneous SCCs after transplantation significantly decreased compared with transplantations in earlier time periods (Table 2).

Factors that characterized the different transplantation time periods are depicted in Table 3. The most important differences were increasing age at transplantation in the later time periods and important differences in immunosuppressive regimens (Table 3).

Patients with cutaneous SCCs are at risk for subsequent IMs
Table 4 shows the distribution of patients without and with cutaneous SCCs before the development of IMs. Of the KTRs with IMs 22% had a prior cutaneous SCCs, whereas only 8% of the patients without IMs had developed cutaneous SCCs (last row of Table 4). Adjustment for age at transplantation, sex, time period of transplantation, and immunosuppressive therapy reduced the hazard ratio from 4 to 3, suggesting that there was partial confounding by these factors for the association between cutaneous SCCs and IMs (Table 4). The analyses stratified for time period of transplantation and immunosuppressive regimen were always in the same direction as the analyses on all KTRs together, which can be used as an argument that the development of cutaneous SCCs is associated with an increased risk of IMs, largely independent of period of transplantation and immunosuppressive regimen (Table 4). It should be noted, however, that, although not statistically significant, the hazard ratios for developing IMs are increasing by transplantation period and that the hazard ratio is much higher in the KTRs immunosuppressed with mycophenolate mofetil (Table 4). The latter observation may indicate that immunosuppression and period of transplantation may also be playing a significant but smaller role. These stratified analyses, however, are hampered by low numbers of events in some of the strata. For example, there were only four patients with a prior cutaneous SCCs in the time period between 1996 and 2006, and only six patients with a prior cutaneous SCCs among the KTRs immunosuppressed with mycophenolate mofetil, resulting in a low statistical power and large confidence intervals (Table 4). There was no statistically significant interaction between the immunosuppressives used and cutaneous SCCs.

Compared with the general population, the risk to develop IMs was 1.6 times increased in all KTRs together (Table 5; Wisgerhof *et al.*, 2011). In KTRs with prior cutaneous SCCs, however, the SMR was much higher, and in KTRs without cutaneous SCCs the SMR was only marginally increased (Table 5). The 29 IMs that developed in the KTRs with prior cutaneous SCCs consisted of 3 head and neck cancers (one oral cavity, one salivary glands, one pharynx), 11 cancers of the digestive organs (two esophagus, four stomach, four colon, one biliary tract), 4 lung cancers, 1 breast cancer, 3 prostate cancers, 6 hematolymphopoetic cancers (three leukemia, three lymphomas), and 1 unknown primary cancer.

DISCUSSION
This study showed a 3-fold increased risk of IMs in KTRs with a prior cutaneous SCCs. The risk of IMs in KTRs without cutaneous SCCs was only marginally increased compared with the general population. The development of cutaneous SCCs may, therefore, be used as an important indicator of increased risk of IMs in these patients.

Table 1. Baseline characteristics of 1,800 kidney transplant recipients without and with cutaneous SCC or internal malignancy

	No malignancy	Cutaneous SCC[1]	Internal malignancy[1]
Number of patients: N	1,521	176	142
Time periods of transplantation: N *(%)*			
1966–1975	168 (11.0)	54 (30.7)	22 (15.5)
1976–1985	315 (20.7)	84 (47.7)	51 (35.9)
1986–1995	429 (28.3)	34 (19.3)	54 (38.0)
1996–2006	609 (40.0)	4 (2.3)	15 (10.6)
Immunosuppressive therapy: N *(%)*			
Aza combination	501 (34.8)	135 (76.7)	77 (54.2)
MMF combination	473 (32.8)	7 (4.0)	8 (5.6)
CyA or Tac	467 (32.4)	34 (19.3)	57 (40.2)
Missing values	80 (5.3%)	0	0
Sex: N *(%)*			
Female	586 (38.5)	57 (32.4)	63 (44.4)
Male	935 (61.5)	119 (67.6)	79 (55.6)
Age at transplantation (years)			
Median (25–75%)	43.2 (32.4–54.8)	39.1 (28.5–47.3)	46.1 (35.1–53.3)
Age at first cancer (years)			
Median (25–75%)	—	58.5 (46.1–64.2)	62.3 (51.3–69.6)
Follow-up after transplantation (years)			
Median (25–75%)	7.7 (3.2–15.1)	22.4 (15.1–27.9)	13.4 (7.6–19.0)
Timing of malignancies			
Only cutaneous SCC	—	137	—
SCC before IM	—	29	29
IM before SCC	—	10	10
Only internal malignancy	—	—	103

Abbreviations: Aza, azathioprine; CyA, cyclosporine A; IM, internal malignancy; MMF, mycophenolate mofetil; SSC, squamous cell carcinoma; Tac, tacrolimus.
[1]The total number of patients is 1,800, but 39 patients have both cutaneous SCC and IMs.
The distributions of these characteristics stratified according to time periods of transplantation are presented in Table 3.

The 3-fold increased risk of IMs in KTRs with cutaneous SCCs is compatible with some studies in the general population, in which a 1.2- to 2.0-fold increased risk of IMs has been reported in patients with prior cutaneous SCCs (Levi et al., 1997; Karagas et al., 1998; Wassberg et al., 1999; Hjalgrim et al., 2000; Hu et al., 2005; Chen et al., 2008; Krueger and Williams, 2010). An inherited predisposition of cancer, a suboptimal immune response, a different level of immunosuppression, or a different immunosuppressive drug regimen, or lifestyle factors (smoking, sun exposure), are possible explanations for the increased risk of IMs in patients with prior cutaneous SCCs (Karagas et al., 1998).

Other studies in the general population, however, have found no or even a negative association between IMs and prior cutaneous SCCs (Levi et al., 1997; de Vries et al., 2007; Grant, 2007; Tuohimaa et al., 2007; Soerjomataram et al., 2008; Cantwell et al., 2009). A mechanism posited to explain this phenomenon involves the connection between sun exposure and vitamin D production in the patients who develop cutaneous SCCs, and a consequent decreased risk of

Table 2. Risk factors for cutaneous SCC and internal malignancy in 1,800 kidney transplant recipients[1]

	Cutaneous SCC		Internal malignancy	
	Non-adjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[2]	Non-adjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[2]
Time periods of transplantation				
1966–1975	1	1	1	1
1976–1985	0.95 (0.67; 1.3)	0.76 (0.53; 1.1)	1.4 (0.82; 2.3)	0.99 (0.58; 1.7)
1986–1995	*0.59 (0.37; 0.92)*	*0.38 (0.21; 0.67)*	2.0 (1.2; 3.5)	1.2 (0.61; 2.2)
1996–2006	*0.23 (0.08; 0.66)*	*0.13 (0.04; 0.46)*	1.3 (0.63; 2.6)	1.1 (0.43; 2.6)
Immunosuppressive therapy				
Aza combination	1	1	1	1
MMF combination	*0.37 (0.17; 0.81)*	0.62 (0.24; 1.6)	0.48 (0.23; 1.0)	*0.27 (0.11; 0.66)*
CyA or Tac	*0.67 (0.45; 0.99)*	0.64 (0.39; 1.0)	1.7 (1.1; 2.4)	0.83 (0.51; 1.3)
Sex				
Female	1	1	1	1
Male	*1.4 (1.0; 2.0)*	*1.7 (1.2; 2.3)*	0.80 (0.57; 1.1)	0.86 (0.62; 1.2)
Age at transplantation (years)				
<30	1	1	1	1
30–39	*1.5 (1.0; 2.3)*	*1.9 (1.2; 2.9)*	*3.0 (1.6; 5.4)*	*3.0 (1.6; 5.6)*
40–49	*2.8 (1.8; 4.2)*	*3.7 (2.4; 5.6)*	*5.0 (2.8; 8.9)*	*5.3 (2.9; 9.7)*
50–59	*2.2 (1.3; 3.9)*	*4.1 (2.3; 7.3)*	*7.7 (4.1; 14.3)*	*7.8 (4.1; 15.0)*
⩾60	*5.5 (2.9; 10.4)*	*15.3 (7.5; 31.2)*	*12.2 (6.1; 24.5)*	*12.9 (6.1; 27.3)*
Increase of risk per 10 years	*1.5 (1.3; 1.7)*	*1.7 (1.5; 2.0)*	*1.8 (1.6; 2.1)*	*1.8 (1.5; 2.1)*

Abbreviations: Aza, azathioprine; CI, confidence interval; CyA, cyclosporine A; MMF, mycophenolate mofetil; Tac, tacrolimus.
[1]The distribution of the different factors is provided in Table 1.
[2]Hazard ratios are adjusted for sex, age at transplantation (as continuous variable), time period of transplantation, and immunosuppressive therapy.
Statistically significant hazard ratios are indicated in italics.

IMs because of the high vitamin D levels in these patients (de Vries *et al.*, 2007; Garland *et al.*, 2009; Krueger and Williams, 2010). However, the reliability of the protective effect of UV-induced high vitamin D levels has been questioned by other researchers (Lindelof *et al.*, 2011).

KTRs with cutaneous SCCs visit their dermatologists usually four times per year and sometimes even more frequently, which is more often than patients without skin cancer. These extra visits may lead to some degree of ascertainment bias, because these KTRs may receive more tests and scrutiny, which may have resulted in a higher rate of IM detection. Dermatologists, however, do not routinely ask for symptoms, which may be related to IMs, and the development of cutaneous SCCs is no reason for their nephrologists to increase the number of routine follow-up visits. It is, therefore, not likely that ascertainment bias explains the increased risk of IM in KTRs with cutaneous SCCs.

An intriguing finding in our study was the impressive drop in risk of cutaneous SCCs in the later transplantation time periods despite the fact that the patients were becoming increasingly older at transplantation, whereas this drop in risk was not observed for IMs. At first sight, the changing immunosuppressive regimen could be responsible for the drop in cancer risk, as immunosuppression with Aza was abandoned after 1986. If immunosuppression would have been the main factor, a similar effect could have been expected for IMs. The later transplantation time periods are also characterized by stringent advices regarding sun avoidance. It is conceivable that avoiding sun exposure strongly decreases the risk of cutaneous SCCs and leaves the risk of IMs unaffected. Yet another possibility would be a lower detection rate of cutaneous SCCs during the later time periods. This option does not appear to be plausible, because almost all KTRs with skin problems from the studied cohort were regularly followed up in our specialized dedicated KTR clinic,

Table 3. Distribution of risk factors during different time periods of transplantation

	1966–1975	1976–1985	1986–1995	1996–2006
Number of patients: *N*	234	433	506	627
Immunosuppressive therapy: N *(%)*				
Aza combination	230 (98.3)	365 (84.3)	88 (17.4)	1 (0.2)
MMF combination	1 (0.4)	2 (0.5)	59 (11.7)	425 (77.6)
CyA or Tac	3 (1.3)	66 (15.2)	358 (70.9)	122 (22.2)
Missing values	0	0	1 (0.2%)	79 (12.6%)
Sex: N *(%)*				
Female	82 (35.0)	180 (41.6)	184 (36.4)	246 (39.2)
Male	152 (65.0)	253 (58.4)	322 (63.6)	381 (60.8)
Age at transplantation (years)				
Median (25–75%)	31.6 (22.5–41.9)	36.6 (26.7–46.9)	46.5 (36.9–56.9)	50.1 (38.6–59.1)
Age at first cutaneous SCC (years)	*N=54*	*N=84*	*N=34*	*N=4*
Median (25–75%)	46.8 (42.1–56.3)	53.4 (46.6–58.6)	60.1 (54.2–65.4)	68.4 (64.9–69.4)
Age at first internal malignancy (years)	*N=22*	*N=51*	*N=54*	*N=15*
Median (25–75%)	54.3 (44.4–61.9)	56.5 (43.9–62.4)	57.8 (53.2–66.7)	60.5 (49.7–71.6)
Follow-up after transplantation (years)				
Median (25–75%)	19.2 (6.2–31.9)	18.5 (10.0–24.4)	12.3 (6.3–15.6)	4.2 (2.1–7.0)

Abbreviations: Aza, azathioprine; CyA, cyclosporine A; MMF, mycophenolate mofetil; SCC, squamous cell carcinoma; Tac, tacrolimus.

dermatologists are currently better trained to recognize this specific problem in KTRs, nephrologists in the Netherlands are educated to refer KTRs with skin problems to a dermatologist at an early stage, and all suspicious lesions were biopsied for a histological diagnosis.

A limitation of the study is that we do not have complete data regarding immunosuppressive drug regimens and switching over time, and that we do not have individualized data for the use of anti-thymocyte globulin or muromonab-CD3 (Orthoclone OKT3) in the KTRs who were transplanted before 1986, and thus we cannot adjust for these potential confounders. In the KTRs who were transplanted in 1986 and later, however, there was no association between the use of anti-thymocyte globulin and/or OKT3 and SCCs (Wisgerhof *et al.*, 2009), and there was also no association with IMs (data not shown). It is possible that immunosuppression and year of transplantation may be playing a significant but smaller role, and the study was underpowered to detect such a difference.

The observation that KTRs show an increased risk of IMs, in particular carcinomas of the digestive organs, lungs, and prostate after the development of cutaneous SCCs, is to our knowledge previously unreported. Therefore, this association

should be validated in other studies. Both nephrologists and dermatologists should be aware of the increased risk of IMs in KTRs with cutaneous SCCs and should be extra careful when skin cancers start to develop in their patients.

MATERIALS AND METHODS

Patients

We performed a cohort study of all 1,906 patients who received a first kidney transplantation at the Leiden University Medical Center (LUMC) between March 1966 and January 2006 (Wisgerhof *et al.*, 2011). The follow-up of the patients ended arbitrarily on 1 June 2007. The study adhered to the Declaration of Helsinki Principles, and the medical ethics committee of the LUMC had approved the study design.

Collection of data

Data recorded for all KTRs 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 IMs and cutaneous SCCs and were collected from the computerized oncological registry of the LUMC, the database from the Department of Pathology and the National Histological Database (PALGA;

Table 4. Risk of internal malignancy in patients with prior cutaneous squamous cell carcinoma

Time period of transplantation immunosuppressive regimen	No internal malignancy	Internal malignancy	Non-adjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[1]
1966–1975				
Squamous cell carcinoma: *N* (%)				
No	168 (79.2)	12 (66.7)	1	1
Yes	44 (20.8)	6 (33.3) (4)[2]	2.9 (1.1; 7.9)	1.9 (0.67; 5.2)
1976–1985				
Squamous cell carcinoma: *N* (%)				
No	315 (82.5)	34 (70.8)	1	1
Yes	67 (17.5)	14 (29.2) (3)[2]	4.1 (2.0; 8.1)	2.8 (1.4; 5.6)
1986–1995				
Squamous cell carcinoma: *N* (%)				
No	429 (94.9)	43 (84.3)	1	1
Yes	23 (5.1)	8 (15.7) (3)[2]	6.1 (2.8; 13.4)	4.1 (1.9; 9.1)
1996–2006				
Squamous cell carcinoma: *N* (%)				
No	609 (99.5)	14 (93.3)	1	1
Yes	3 (0.5)	1 (6.7) (0)[2]	10.9 (1.3; 90.2)	6.5 (0.70; 60.4)
Azathioprine combination				
Squamous cell carcinoma: *N* (%)				
No	501 (82.5)	48 (68.6)	1	1
Yes	106 (17.5)	22 (31.4) (7)[2]	4.6 (2.7; 8.1)	3.0 (1.7; 5.2)
Mycophenolate mofetil combination				
Squamous cell carcinoma: *N* (%)				
No	473 (98.7)	7 (87.5)	1	1
Yes	6 (1.3)	1 (12.5) (0)[2]	69.0 (6.0; 786.0)	37.1 (2.6; 543.5)
Cyclosporine and tacrolimus combination				
Squamous cell carcinoma: *N* (%)				
No	467 (94.9)	48 (88.9)	1	1
Yes	25 (5.1)	6 (11.1) (3)[2]	2.7 (1.1; 6.7)	2.1 (0.88; 5.2)
All patients together				
Squamous cell carcinoma: *N* (%)				
No	1,521 (91.7)	103 (78.0)	1	1
Yes	137 (8.3)	29 (22.0) (10)[2]	4.3 (2.7; 6.8)	3.0 (1.9; 4.7)

[1]The hazard ratio for all patients together was adjusted for sex, age at transplantation, time period of transplantation, and immunosuppressive therapy. The hazard ratios in the different strata of time after transplantation and immunosuppressive regimen were adjusted for age and sex.
[2]Number of patients who were censored because the event (internal malignancy) occurred before the development of cutaneous squamous cell carcinoma.

Wisgerhof *et al.*, 2011). The medical charts were also hand searched for the diagnosis of cancer. Premalignant lesions and *in situ* carcinomas were not included. KTRs who were lost to follow-up at the first transplantation, who already had cancer before the first transplantation, and patients with cutaneous basal cell carcinoma or other types of skin cancer (malignant melanoma, Kaposi's sarcoma, sweat gland carcinoma, and so on) without cutaneous SCCs or IMs were excluded from all analyses (Figure 1).

Table 5. SMRs adjusted for age, sex, and time period for different types of cancer in kidney transplant recipients restricted to malignancies that developed after transplantation and stratified according to patients with and without prior cutaneous SCC

	Internal malignancies observed	Internal malignancies expected	SMR (95% CI)
All patients together (*N*=1,800)	142	89.43	1.6 (1.3; 1.9)
Patients with prior SCC (*N*=166)	29	8.24	3.5 (2.4; 5.0)
Patients without prior SCC (*N*=1,624)	103	80.60	1.3 (1.0; 1.5)

Abbreviations: CI, confidence interval; SCC, squamous cell carcinoma; SMRs, standardized morbidity ratios.

The diagnoses of IMs 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 SCCs and not as IMs.

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 as described before (Wisgerhof *et al.*, 2011). The time period of transplantation was arbitrarily stratified into 1966–1975; 1976–1985; 1986–1995; and 1996–2006.

Immunosuppressive regimens

Between 1966 and 1986, the immunosuppressive treatment of KTRs in our clinic consisted of duo therapy with prednisolone and Aza, but shortly after 1986 all new KTRs were immunosuppressed with prednisolone and CyA. From the mid 90s, most KTRs were treated with prednisolone, mycophenolate mofetil (MMF), and CyA, and later with prednisolone, MMF, and tacrolimus. 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 (that is, a combination of prednisolone with CyA or prednisolone with tacrolimus). If no data were available for 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.

KTRs, 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 anti-thymocyte globulin and a third rejection treatment again with methylprednisolone were given. In exceptional cases, muromonab-CD3 (Orthoclone OKT3) was given when a fourth rejection treatment was needed. With the exception of some rare patients, induction treatments with anti-thymocyte globulin and/or OKT3 were not given to KTRs who were transplanted in the LUMC between 1966 and 1995.

Statistical analyses

For statistical analyses, we used χ^2 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 IMs or cutaneous SCCs and to adjust for potentially confounding factors. As potential confounding factors, we selected age at transplantation and sex, because these factors have been reported to be associated with both IMs and cutaneous SCCs. The time period of transplantation and the immunosuppressive therapy were also selected as potential confounding factors, because these factors also appeared to be associated with IMs or cutaneous SCCs.

In traditional Cox regression analysis, a risk factor measured at baseline is related to the event thereafter. During follow-up, however, things may change: either the effect of a fixed baseline risk factor may vary over time, resulting in a weakening or strengthening of associations over time, or the risk factor itself may vary over time. The development of cutaneous SCCs, when using IMs as the outcome, is an example of the latter. Time-dependent Cox regression analyses were used to measure the effect of this so-called time-dependent risk factor. As opening dates for the analyses, we used the date of the first transplantation; as closing dates we used the date of diagnosis of the IM, the date of the patient's death, or the date of last follow-up. Patients were not censored from the analyses at graft failure.

The incidence of IMs in the KTRs after transplantation was compared with the incidence in the general population, the data for which were obtained from the Eindhoven Cancer Registry and the Netherlands Cancer Registry by calculating the SMR with 95% confidence interval and was matched for age, sex, and time period in which the malignancy had occurred (Wisgerhof *et al.*, 2011).

P-values below 0.05 were considered significant. All statistical calculations were performed using SPSS for Windows version 17 (SPSS, Chicago, IL).

REFERENCES

Adami J, Gabel H, Lindelof B *et al.* (2003) Cancer risk following organ transplantation: a nationwide cohort study in Sweden. *Br J Cancer* 89:1221–7

Alam M, Brown RN, Silber DH *et al.* (2011) Increased incidence and mortality associated with skin cancers after cardiac transplant. *Am J Transplant* 11:1488–97

Cantwell MM, Murray LJ, Catney D *et al.* (2009) Second primary cancers in patients with skin cancer: a population-based study in Northern Ireland. *Br J Cancer* 100:174–7

Chen J, Ruczinski I, Jorgensen TJ *et al.* (2008) Nonmelanoma skin cancer and risk for subsequent malignancy. *J Natl Cancer Inst* 100:1215–22

de Vries E, Soerjomataram I, Houterman S *et al.* (2007) Decreased risk of prostate cancer after skin cancer diagnosis: a protective role of ultraviolet radiation? *Am J Epidemiol* 165:966–72

Euvrard S, Kanitakis J, Claudy A (2003) Skin cancers after organ transplantation. *N Engl J Med* 348:1681–91

Euvrard S, Kanitakis J, Decullier E *et al.* (2006) Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. *Transplantation* 81:1093–100

Garland CF, Gorham ED, Mohr SB *et al.* (2009) Vitamin D for cancer prevention: global perspective. *Ann Epidemiol* 19:468–83

Grant WB (2007) A meta-analysis of second cancers after a diagnosis of nonmelanoma skin cancer: additional evidence that solar ultraviolet-B irradiance reduces the risk of internal cancers. *J Steroid Biochem Mol Biol* 103:668–74

Grulich AE, van Leeuwen MT, Falster MO *et al.* (2007) Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 370:59–67

Hartevelt MM, Bouwes Bavinck JN, Kootte AM *et al.* (1990) Incidence of skin cancer after renal transplantation in The Netherlands. *Transplantation* 49:506–9

Hjalgrim H, Frisch M, Storm HH *et al.* (2000) Non-melanoma skin cancer may be a marker of poor prognosis in patients with non-Hodgkin's lymphoma. *Int J Cancer* 85:639–42

Hu S, Federman DG, Ma F *et al.* (2005) Skin cancer and non-Hodgkin's lymphoma: examining the link. *Dermatol Surg* 31:76–82

Karagas MR, Greenberg ER, Mott LA *et al.* (1998) Occurrence of other cancers among patients with prior basal cell and squamous cell skin cancer. *Cancer Epidemiol Biomarkers Prev* 7:157–61

Krueger H, Williams D (2010) Burden of malignancy after a primary skin cancer: recurrence, multiple skin cancers and second primary cancers. *Cancer J Public Health* 101:I23–7

Levi F, Randimbison L, La VC *et al.* (1997) Incidence of invasive cancers following squamous cell skin cancer. *Am J Epidemiol* 146: 734–9

Lindelof B, Krynitz B, Ayoubi S *et al.* (2011) Previous extensive sun exposure and subsequent vitamin D production in patients with basal cell carcinoma of the skin, has no protective effect on internal cancers. *Eur J Cancer*, July 23

London NJ, Farmery SM, Will EJ *et al.* (1995) Risk of neoplasia in renal transplant patients. *Lancet* 346:403–6

Soerjomataram I, Louwman WJ, Lemmens VE *et al.* (2008) Are patients with skin cancer at lower risk of developing colorectal or breast cancer? *Am J Epidemiol* 167:1421–9

Tuohimaa P, Pukkala E, Scelo G *et al.* (2007) Does solar exposure, as indicated by the non-melanoma skin cancers, protect from solid cancers: vitamin D as a possible explanation. *Eur J Cancer* 43:1701–12

Vajdic CM, McDonald SP, McCredie MR *et al.* (2006) Cancer incidence before and after kidney transplantation. *JAMA* 296:2823–31

Villeneuve PJ, Schaubel DE, Fenton SS *et al.* (2007) Cancer incidence among Canadian kidney transplant recipients. *Am J Transplant* 7:941–8

Wassberg C, Thorn M, Yuen J *et al.* (1999) Second primary cancers in patients with squamous cell carcinoma of the skin: a population-based study in Sweden. *Int J Cancer* 80:511–5

Wisgerhof HC, Edelbroek JR, de Fijter JW *et al.* (2010) Subsequent squamous- and basal-cell carcinomas in kidney-transplant recipients after the first skin cancer: cumulative incidence and risk factors. *Transplantation* 89:1231–8

Wisgerhof HC, van der Boog PJ, de Fijter JW *et al.* (2009) Increased risk of squamous-cell carcinoma in simultaneous pancreas kidney transplant recipients compared with kidney transplant recipie. *J Invest Dermatol* 129:2886–94

Wisgerhof HC, van der Geest LG, de Fijter JW *et al.* (2011) Incidence of cancer in kidney-transplant recipients: A long-term cohort study in a single center. *Cancer Epidemiol* 35:105–11