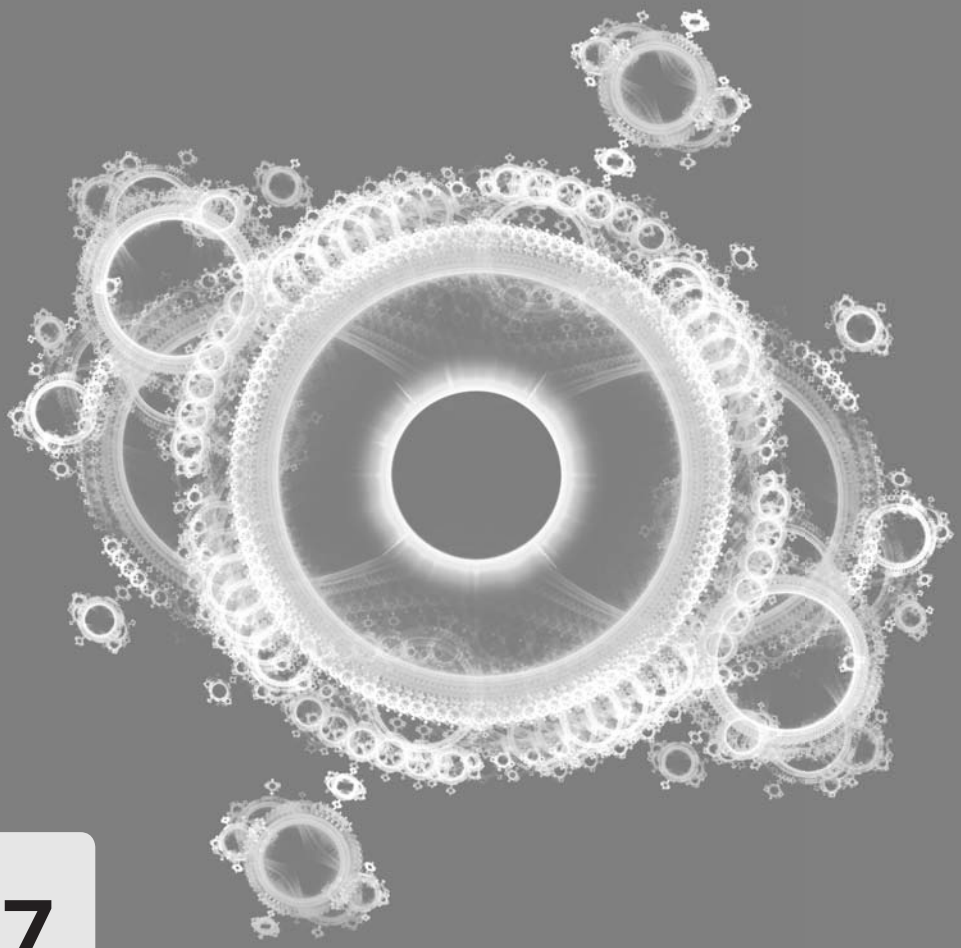
Epidemiogic aspects of skin cancer in organ-transplant recipients

Wisgerhof, H.C.



7

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

Hermina C. Wisgerhof • Ron Wolterbeek • Geert W. Haasnoot • Frans H.J. Claas
Johan W. de Fijter • Rein Willemze • Jan N. Bouwes Bavinck

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.

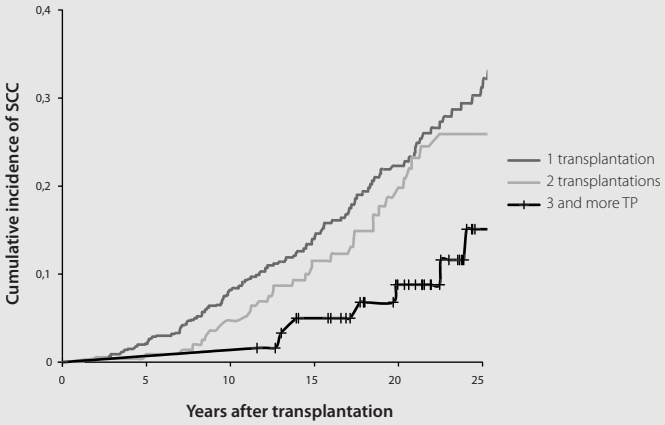
Table 4 Risk factors of cancer in the kidney-transplant recipients.

	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)...

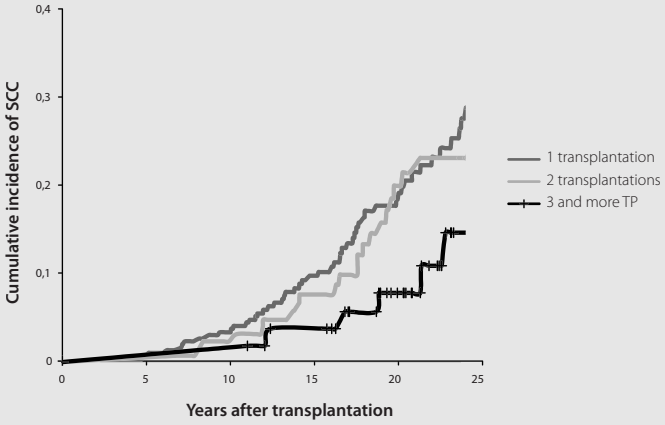
A All patients



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

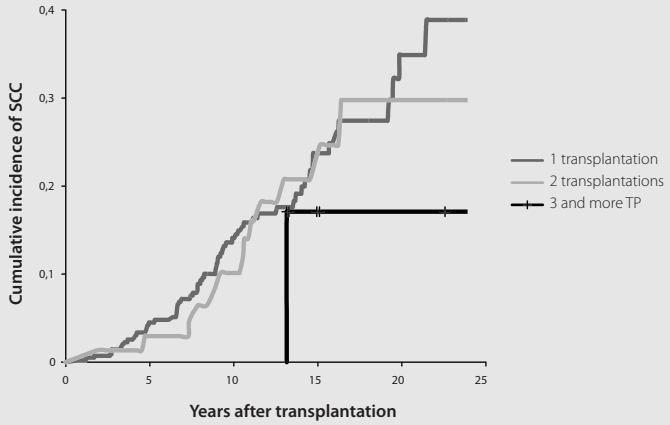


Number of patients at risk

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),...

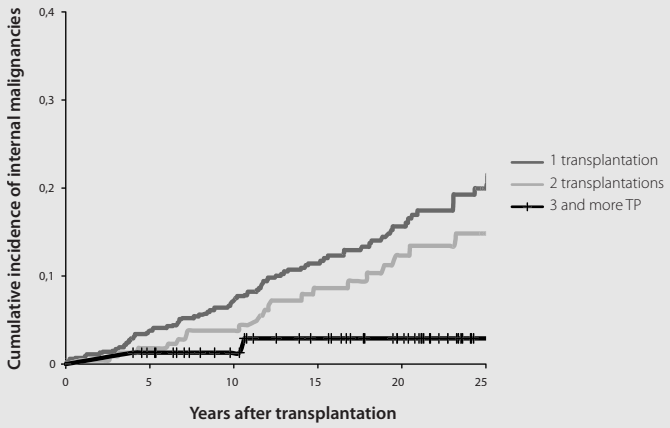
C Patients of 40 years and older at transplantation



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

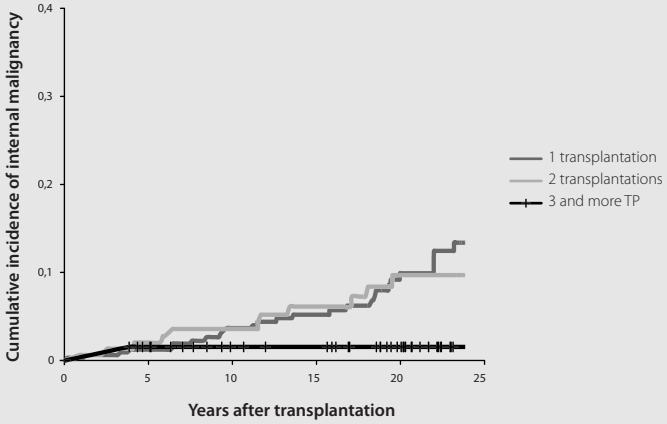


Number of patients at risk

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).

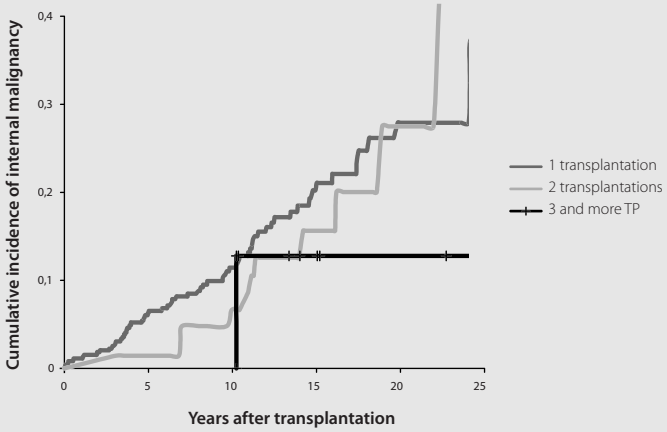
E Patients younger than 40 years at transplantation



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.

Reference List

1. Wisgerhof HC, van der Geest LG, de Fijter JW et al. Incidence of cancer in kidney-transplant recipients: A long-term cohort study in a single center. Cancer Epidemiol 2010.
2. Bordea C, Wojnarowska F, Millard PR, Doll H, Welsh K, Morris PJ. Skin cancers in renal-transplant recipients occur more frequently than previously recognized in a temperate climate. Transplantation 2004;77: 574-579.
3. Bouwes Bavinck JN, Hardie DR, Green A et al. The risk of skin cancer in renal transplant recipients in Queensland, Australia. A follow-up study. Transplantation 1996;61: 715-721.
4. Euvrard S, Kanitakis J, Pouteil-Noble C, Claudy A, Touraine JL. Skin cancers in organ transplant recipients. Ann Transplant 1997;2: 28-32.
5. Hartevelt MM, Bavinck JN, Kootte AM, Vermeer BJ, Vandenbroucke JP. Incidence of skin cancer after renal transplantation in The Netherlands. Transplantation 1990;49: 506-509.
6. Moloney FJ, Comber H, O'Lorcain P, O'Kelly P, Conlon PJ, Murphy GM. A population-based study of skin cancer incidence and prevalence in renal transplant recipients. Br J Dermatol 2006;154: 498-504.
7. Hardie IR, Strong RW, Hartley LC, Woodruff PW, Clunie GJ. Skin cancer in Caucasian renal allograft recipients living in a subtropical climate. Surgery 1980;87: 177-183.
8. Kasiske BL, Snyder JJ, Gilbertson DT, Wang C. Cancer after kidney transplantation in the United States. Am J Transplant 2004;4: 905-913.
9. Villeneuve PJ, Schaubel DE, Fenton SS, Shepherd FA, Jiang Y, Mao Y. Cancer incidence among Canadian kidney transplant recipients. Am J Transplant 2007;7: 941-948.
10. Wisgerhof HC, van der Boog PJ, de Fijter JW et al. Increased Risk of Squamous-Cell Carcinoma in Simultaneous Pancreas Kidney Transplant Recipients Compared with Kidney Transplant Recipients. J Invest Dermatol 2009;129: 2886-2894.
11. Naldi L, Fortina AB, Lovati S et al. Risk of nonmelanoma skin cancer in Italian organ transplant recipients. A registry-based study. Transplantation 2000;70: 1479-1484.
12. Ramsay HM, Fryer AA, Hawley CM, Smith AG, Harden PN. Non-melanoma skin cancer risk in the Queensland renal transplant population. Br J Dermatol 2002;147: 950-956.
13. Ramsay HM, Fryer AA, Hawley CM, Smith AG, Nicol DL, Harden PN. Factors associated with nonmelanoma skin cancer following renal transplantation in Queensland, Australia. J Am Acad Dermatol 2003;49: 397-406.
14. van Leeuwen MT, Grulich AE, McDonald SP et al. Immunosuppression and other risk factors for lip cancer after kidney transplantation. Cancer Epidemiol Biomarkers Prev 2009;18: 561-569.
15. Ingvar A, Smedby KE, Lindelof B et al. Immunosuppressive treatment after solid organ transplantation and risk of post-transplant cutaneous squamous cell carcinoma. Nephrol Dial Transplant 2009.
16. House AA, Chang PC, Luke PP et al. Re-exposure to mismatched HLA class I is a significant risk factor for graft loss: multivariable analysis of 259 kidney retransplants. Transplantation 2007;84: 722-728.
17. van Kampen CA, Versteeg-van der Voort Maarschalk MF, Roelen DL, ten B, I, Claas FH. Rejection of a kidney transplant does not always lead to priming of cytotoxic T cells against mismatched donor HLA class I antigens. Transplantation 2001;71: 869-874.
18. Joosten SA, Sijpkens YW, van KC, Paul LC. Chronic renal allograft rejection: pathophysiologic considerations. Kidney Int 2005;68: 1-13.
19. Bresnahan BA, Johnson CP, McIntosh MJ, Stablein D, Hariharan S. A comparison between recipients receiving matched kidney and those receiving mismatched kidney from the same cadaver donor. Am J Transplant 2002;2: 366-372.
20. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000;342: 605-612.
21. Goldfarb-Rumyantzev AS, Naiman N. Genetic prediction of renal transplant outcome. Curr Opin Nephrol Hypertens 2008;17: 573-579.
22. Kruger B, Schroppel B, Murphy BT. Genetic polymorphisms and the fate of the transplanted organ. Transplant Rev (Orlando) 2008;22: 131-140.
23. Kainz A, Korbely R, Soleiman A, Mayer B, Oberbauer R. Antithymocyte globulin use for treatment of biopsy confirmed acute rejection is associated with prolonged renal allograft survival. Transpl Int 2010;23: 64-70.

24. Macdonald FI, Ashraf S, Picton M et al. Banff criteria as predictors of outcome following acute renal allograft rejection. Nephrol Dial Transplant 1999;14: 1692-1697.
25. Caforio AL, Fortina AB, Piaserico S et al. Skin cancer in heart transplant recipients: risk factor analysis and relevance of immunosuppressive therapy. Circulation 2000;102: III222-III227.
26. Fortina AB, Piaserico S, Caforio AL et al. Immunosuppressive level and other risk factors for basal cell carcinoma and squamous cell carcinoma in heart transplant recipients. Arch Dermatol 2004;140: 1079-1085.
27. Jensen P, Moller B, Hansen S. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol 2000;42: 307.
28. Ferrandiz C, Fuente MJ, Ribera M et al. Epidermal dysplasia and neoplasia in kidney transplant recipients. J Am Acad Dermatol 1995;33: 590-596.
29. Fortina AB, Caforio AL, Piaserico S et al. Skin cancer in heart transplant recipients: frequency and risk factor analysis. J Heart Lung Transplant 2000;19: 249-255.
30. Harden PN, Fryer AA, Reece S, Smith AG, Ramsay HM. Annual incidence and predicted risk of non-melanoma skin cancer in renal transplant recipients. Transplant Proc 2001;33: 1302-1304.
31. Adams AB, Pearson TC, Larsen CP. Heterologous immunity: an overlooked barrier to tolerance. Immunol Rev 2003;196: 147-160.
32. Amir AL, D'Orsogna LJ, Roelen DL et al. Allo-HLA reactivity of virus-specific memory T cells is common. Blood 2010;115(15): 3146-3157.
33. Salama AD, Womer KL, Sayegh MH. Clinical transplantation tolerance: many rivers to cross. J Immunol 2007;178: 5419-5423.