Title: Epithelial Skin cancers after kidney transplantation: a retrospective single-centre study on 376 recipients

Short title: Non melanoma skin cancer in renal transplant recipients

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Abstract

Background: Post-transplant non-melanoma skin cancers (NMSC) are the most common malignancies in kidney transplant recipients.

Objective: To analyse risk factors associated with the occurrence of basal cell carcinomas (BBC) and squamous cell carcinomas (SCC).

Patients and Methods: Statistical analysis was performed on 376 kidney transplant recipients screened NMSC in the years 2002-2009 and followed-up until 2013.

Results: NMSC developed in 23.67% of individuals with an SCC/BCC ratio of 2.15 : 1 and an age-standardized incidence ratio (IR) of 2.71 cases (95% CI, 1.97 - 3.46) per 100 patients/year. In multivariable analysis NMSC occurrence significantly correlated with higher age ($p<0.001$), fair skin type ($p = 0.01$) and particularly in SCCs with male gender ($p = 0.001$). Patients with >10 AKs were at higher risk to develop NMSCs (IRR = 2.95; 95% CI, 1.97 – 4.42; $p < 0.001$) and more prone to SCCs as compared to BCCs ($p = 0.04$). Also, more SCC carriers had high counts of warty lesions ($p = 0.006$). Calcineurin-inhibitors were associated with higher NMSC incidence (IRR = 2.81; 95% CI, 1.13 - 7.01; $p = 0.03$), while no difference was seen with mammalian target of Rapamycin (mTOR)-inhibitors.

Conclusions: In addition to the duration of immunosuppression our results further confirm an influence of the individual immunosuppressive regimen and suggest that older patients, males, fair skinned recipients or those affected with high AK counts (field cancerization) are particularly prone to NMSC development.

Key words: immunosuppression, kidney transplant recipients, non-melanoma skin cancer

Introduction

Non-melanoma skin cancer (NMSC) is the most common type of neoplasm in humans and also the malignant disease that is increasingly prevalent among organ transplant recipients (OTRs) due to long-term immunosuppressive therapy and other factors [1]. Tumours can be more aggressive in OTRs than in the general population with a rapid progression and destructive behaviour, resulting in significantly higher morbidity and mortality [2]. In contrast to the immunocompetent population, skin cancers in OTRs are dominated by squamous cell carcinoma (SCC), followed by basal cell carcinoma (BCC). In fact, transplanted individuals of Caucasian Origin have a 60-to 250-fold increased risk for SCC and a 10-fold increased risk for BCC, while for malignant melanoma the incidence appears only 2-3 folds higher than in the general population [3, 4]. SSCs develop predominantly in areas of field cancerization with multiple actinic keratosis (AK) or Bowen disease and their risk of metastases is increased to approximately 8% for OTRs as compared to 0.5% - 5% in the general population [5]. Moreover, SCCs and BCCs do occur earlier in OTRs as compared to immunocompetent controls and apparently their cumulative incidence rises linearly without a delay after transplantation with a decreasing time interval for subsequent tumours [6].

Apart from immunosuppression other potential co-factors contributing to the augmented likelihood for evolving NMSCs in this patient group have to be considered. Among better defined co-stimuli, indicative of an increased NMSC risk in OTRs patients, there are ultraviolet radiation exposure, male sex, fair skin, presence of keratotic skin lesions, age at transplant or duration and extent of the immunosuppression therapy [7, 8]. In addition, the choice of immunosuppressive agents may be relevant to explain an augmented risk in these patients [9].

After an initial report from a previous multivariable analysis in 243 renal transplant patients, seen by our group until 2005 [10], the purpose of this study was to further extent and validate the parameters determining the occurrence of SCCs and BCCs in our retrospective cohort of 376 kidney transplant recipient and to better define the potential impact of immunosuppressive drugs administered to these patients.

Materials and Methods

Patient cohort

Data from 376 patients who underwent renal transplantation between May 1970 and January 2009 were retrospectively collected and follow-up examinations reported until end of 2013. Starting as of 2002 all patients were examined at our special dermatological consultation unit at the department of nephrology in accordance with internal guidelines of the University of Bern and screened for the occurrence of new NMSC and respective premalignant lesions. The study was approved by the local ethical committee and all patients gave written informed consent. Prior to kidney transplantation all patients had been checked by a dermatologist for skin cancers and/or premalignant conditions and treated for evident lesions.

Apart from patient specific characteristics at study entrance (Age, sex, skin type, duration of immunosuppression, type of immunosuppression) the following parameters were documented and followed in addition at each visit: all clinically evident skin lesions suspect for actinic keratosis (AK), SCC or BCC, as well as the type and dosage of immunosuppression. If a lesion was clinically clearly recognised as AK, treatment was performed without histological confirmation. In all other cases a punch biopsy was evaluated histologically.

Statistical Analysis

For descriptive purpose data are presented as means and standard deviations (SD) or numbers with percentages for continuous and categorical variables respectively. Incidence of first NMSC was computed as incidence rate (IR) per 100 patients/year, together with its 95% confidence interval (CI), by assuming a Poisson distribution. Age-standardized IR was calculated by taking the age structure of the 2000 EU population as reference. For graphical purpose cumulative incidence rates were computed by means of Kaplan-Meier estimator. Pearson's χ^2 test was used to test for difference between categorical variables.

Multiple Poisson regression analysis, including terms for age and gender, was used to assess which factors influenced the incidence of first NMSC in the first 20 years of immunosuppression. Effects of selected factors were expressed as incidence rates ratios

(IRR) along with their 95% CI and p-value. Robust standard errors of parameter estimates were computed to control for mild violation of equal mean and variance assumption. This was also tested by considering a ratio of the deviance and the degree of freedom of its distribution lower than 1. A continuity correction was applied to univariate Poisson model in case of zero entries in one stratum. Analyses were carried out with SPSS ver. 20 (IBM Corp). All tests were considered significant at p-value <0.05.

Results

Patient characteristics are summarized in table 1. The mean age of patients at transplantation was 44.1 ± 14.6 years (mean ± SD), with a male : female ratio of 1.63 : 1. Most of them had a skin type of I or II (54.0%) according to Fitzpatrick types I-VI. Overall the number of AK lesions at study entry was between 1 - 10 for 39.2% of patients and over 10 for 6.9% of them. The mean immunosuppressive treatment duration was 10.6 ± 7.4 years including all follow-up visits (mean number of consultations 3.54 ± 3.43). A mean number of 2.54 ± 0.6 immunosuppressive drugs were combined in 22 different regimens. Overall the most prescribed treatments were prednisone (89.3%) and cyclosporine (81.9%), followed by MMF (35.5%) and azathioprine (32.0%).

Among our 233 male and 143 female renal transplant recipients a total of 89 individuals (23.67%) developed at least one NMSC (Figure 1a). The mean duration until the detection of the first skin cancer was 12.56 ± 7.6 years. The age-standardized IR was 2.71 cases (95% CI, 1.97 - 3.46) per 100 patients/year for NMSC, 1.76 cases (95% CI, 1.19 - 2.33) for BCC and 1.76 cases (95% CI, 1.17 - 2.36) for SCC (Table 2). Overall 358 NMSCs (249 SCCs, 109 BCCs) were observed since first visit corresponding to an average of 4.0 skin cancers per tumour patient. 30 Patients (8.0% from study group or 33.7% from the tumour bearing subgroup) had both SCCs and BBCs, while 27 (7.2 / 30.3 %) had only SCCs and another 32 individuals were recorded with BCCs only (8.5 / 35.9 %). The SCC/BCC ratio was 2.15 : 1 in our series.

According to multivariable analysis in the first 20-years of immunosuppression (Table 3), males were more likely than females to develop a NMSC (IRR = 1.74; 95% CI, 1.11 - 2.73) and in particular a SCC (IRR = 3.30; 95% CI, 1.64 - 6.64). Age had also a significant impact on NMSC incidence, with a clear trend towards higher age groups (Figure 1b). Patients with >10

AKs (defined as field cancerisation) were at higher risk to develop NMSCs (IRR = 2.95; 95% CI, 1.97 – 4.42; p < 0.001). For SCCs the IRR was 5.58 (95% CI, 3.30 – 9.44; p < 0.001) and for BCCs 2.51 (95% CI, 1.47 – 4.29; p = 0.001). The risk for SCCs was higher as compared to the occurrence of BCCs (p = 0.04). Correspondingly, in SCC carriers significantly more individuals had associated high counts of warty lesions (61.4%) as compared to patients with only BCCs (31.2%) (p = 0.006). A skin type of I-II was linked with an increased risk of NMSC (IRR = 1.82; 95% CI, 1.13 - 2.94) and in particular of BCC (IRR = 2.34; 95% CI; 1.23 - 4.45). Among the prescribed drugs, only cyclosporine/tacrolimus showed an association with NMSC incidence (IRR = 2.81; 95% CI, 1.13 - 7.01), while in our patients there was no significant impact with the introduction of sirolimus/everolimus (p = 0.10). Figures 2 a and b display the influence of different medications on the cumulative incidence rates of NMSC since transplantation.

Discussion

Considering the success rates in kidney grafting together with an ongoing demand, we are facing a steadily increasing number of long-term renal transplant survivors. Given the high incidence and aggressive biologic behaviour of NMSC in affected recipients, early detection and removal of NMSCs and respective precursor lesions has become a major challenge. The group screened for skin cancer in this study was predominantly composed of male recipients and our data confirmed an increased risk for developing mainly SCCs. This gender difference reflects data frequently reported by other studies with less females being affected by end-stage renal disease, while in some countries a still persisting gender discrimination in benefiting from medical treatment might explain a more pronounced impact [11]. The association of male gender with a higher incidence of potentially metastatic SCCs makes male kidney recipients an even more sensitive target group for education efforts, regular skin examinations and preventive measures.

Epidemiological studies have demonstrated NMSCs to be the most common malignancies arising after organ transplantation in Caucasians [12] and a recent Swedish population based investigation confirmed these results, with the bulk of the excess hazard driven by an exceptionally high and accelerating risk of SCC [13]. Our current data, with 23.67% of patients developing NMSCs after a mean of 12.56 ± 7.6 years post-transplantation are in accordance with those reported from other cohort studies. Indeed, the incidence of NMSC in the US-

and western European recipient population increases from 10% to 27% at 10 years and from 40% to 60% at 20 years post-transplant, compared to the highest incidence reported in Australia, which is even 80% at 20 years [5]. In contrast, latest data from Korean and Chinese transplant centres further approved a dissimilar malignancy pattern in Asian organ recipients. Whereas the risk of several cancer types is higher than that in the general population, even in long-term follow-ups the cumulative incidence of post-transplantation skin cancer remains extremely low as compared to populations from western countries [14,15].

Consequently, the burden of NMSCs contributes to a substantial morbidity in OTRs of Caucasian origin. In a retrospective analysis of the Irish Renal Transplant Database of 255 patients with ultra-long-term (>20 year) renal allograft survival skin cancer was by far the leading comorbidity (36.1%), followed by coronary heart disease (17.3%) and other malignancies (14.5%) [16]. Accordingly, NMSCs add to a substantial economic load of health care costs in renal graft recipients and may even be responsible for post-transplant mortality[17]. At least, in Australian liver and cardiothoracic transplant recipients de novo cancer was a leading cause of late death, with the highest relative risk due to NMSC [18].

Thus, apart from screening awareness and a close collaboration of renal transplant teams and dermatologists, in particular photo-protection, skin self-examination or a change in immunotherapy were suggested as effective measures against NMSC development in such patients [19]. Primarily, a preventive and aggressive treatment of actinic keratosis in areas of field cancerization is recommended to cut down on the high morbidity and avoid the mortality associated with invasive squamous cell carcinoma in OTRs [2].

Also a conversion of immunosuppressive drugs to m-TOR-inhibitors has been demonstrated to reduce the development of new AKs and NMSCs in several studies as reviewed in a recent meta-analysis in 5876 subjects from 21 randomized trials [20]. According to Karayannopoulou and coworkers the considerably higher expression of phospho-mTOR in SCCs compared to -BCCs might explain their higher sensitivity to mTOR-inhibitors [21]. In our study, among 33 patients (7.95%) receiving sirolimus on 1st visit only two subjects developed BCCs while no SCC could be observed. Yet, no difference could be demonstrated as compared to patients without mTOR-inhibitors probably due to a lack of power in our cohort.

In summary, our current data suggest older patients, males, fair skinned recipients, those presenting multiple warty lesions or field cancerisation to be a particular high-risk group for NMSCs among renal transplant recipients that should probably benefit best from a close follow-up and active surveillance.

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Table 2 NMSC incidence rates x 100 patients/year, overall and by age groups

Table 3 Multivariable analysis of factors influencing NMSC incidence rates during the first 20 years of immunosuppression

Figure 1 NMSC cumulative incidence rates since transplantation, overall (a) and by age groups (b)

Figure 2 NMSC cumulative incidence rates in the first 20 years after transplantation, by use of Cyclosporine/Tacrolimus (a) and Sirolimus/Everolimus (b)

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Tables and Figures

Table 1 Characteristics of 376 renal transplanted patients included in the study

		N*=376	%	Mean	SD
Gender	Male	233	62.0%		
	Female	143	38.0%		
Age at transplantation (years)				44.1	14.6
Skin Type	I - II	157	54.0%		
	III	114	39.2%		
	IV - VI	20	6.9%		
Number of AK lesions	0	246	66.0%		
	1 - 10	83	22.3%		
	>10	44	11.8%		
Age at first visit (years)				51.2	13.2
Number of visits				3.5	3.4
Follow-up (years)				10.6	7.4
Immunosuppressant Drugs	Prednisone	335	89.3%		
	Cyclosporine	307	81.9%		
	MMF	133	35.5%		
	Azathioprin	120	32.0%		
	Tacrolimus	21	5.6%		
	Sirolimus/Everolimus	33	8.8%		
Number of combined drugs				2.5	0.6

SD = standard deviation, AK: actinic keratosis

* Numbers may not add up to the total due to missing data

Table 2 - NMSC incidence rates x 100 patients/year, overall and by age groups

	N*	Patients/year	IR (95% CI)	Age standardized IR (95% CI)
NMSC	**89**	**3672.58**	**2.42 (1.92 - 2.93)**	**2.71 (1.97 - 3.46)**
Age<35	19	1411.68	1.32 (0.73 - 1.91)	
Age 35-49	34	1333.38	2.55 (1.69 - 3.41)	
Age 50+	35	927.52	3.77 (2.52 - 5.02)	
BCC	**62**	**3764.83**	**1.65 (1.24 - 2.06)**	**1.76 (1.19 - 2.33)**
Age<35	15	1431.27	1.05 (0.52 - 1.58)	
Age 35-49	21	1375.00	1.53 (0.87 - 2.18)	
Age 50+	25	958.56	2.61 (1.59 - 3.63)	
SCC	**57**	**3775.83**	**1.51 (1.12 - 1.90)**	**1.76 (1.17 - 2.36)**
Age<35	10	1435.90	0.70 (0.26 - 1.13)	
Age 35-49	24	1364.04	1.76 (1.06 - 2.46)	
Age 50+	23	975.89	2.36 (1.39 - 3.32)	

N = number of cases, CI = confidence interval, IR = incidence rate

* Numbers may not add up to the total due to missing data

Table 3 Multivariable analysis of factors influencing NMSC incidence rates during the first 20 years of immunosuppression

	NMSC		BCC		SCC	
	IRR (95% CI)*	p-value	IRR (95% CI)*	p-value	IRR (95% CI)*	p-value
Gender						
Male	1.74 (1.11 - 2.73)	0.02	1.28 (0.75 - 2.21)	0.37	3.30 (1.64 - 6.64)	0.001
Female	1		1		1	
Age (yrs)						
<35	1		1		1	
35 – 49	2.53 (1.33 - 4.81)	0.005	1.73 (0.80 - 3.73)	0.17	6.56 (2.06 - 20.93)	0.001
50+	4.70 (2.49 - 8.88)	<0.001	3.73 (1.80 - 7.74)	<0.001	11.25 (3.55 - 35.66)	<0.001
Skin type						
I – II	1.82 (1.13 - 2.94)	0.01	2.34 (1.23 - 4.45)	0.01	1.58 (0.86 - 2.92)	0.14
III – VI	1		1		1	
Number of AK lesions						
≤ 10	1		1		1	
>10	2.95 (1.97 - 4.42)	<0.001	2.51 (1.47 - 4.29)	0.001	5.58 (3.30 - 9.44)	<0.001
Drugs						
Prednison						
No	1		1		1	
Yes	1.71 (0.79 - 3.68)	0.17	1.93 (0.63 - 5.85)	0.25	1.26 (0.52 - 3.04)	0.60
Ciclosporin/Tacrolimus						
No	1		1		1	
Yes	2.81 (1.13 - 7.01)	0.03	2.39 (0.80 - 7.18)	0.12	1.64 (0.66 - 4.07)	0.29
MMF						
No	1		1		1	
Yes	0.93 (0.56 - 1.53)	0.76	0.83 (0.43 - 1.61)	0.58	0.66 (0.32 - 1.36)	0.26
Azathioprin						
No	1		1		1	
Yes	1.29 (0.85 - 1.96)	0.23	1.19 (0.69 - 2.07)	0.52	1.63 (0.95 - 2.78)	0.08
Sirolimus/Everolimus						
No	5.11 (0.71 - 36.63)	0.10	3.42 (0.48 - 24.47)	0.22	7.46 (0.70 - 79.63)^	0.10
Yes	1		1		1	

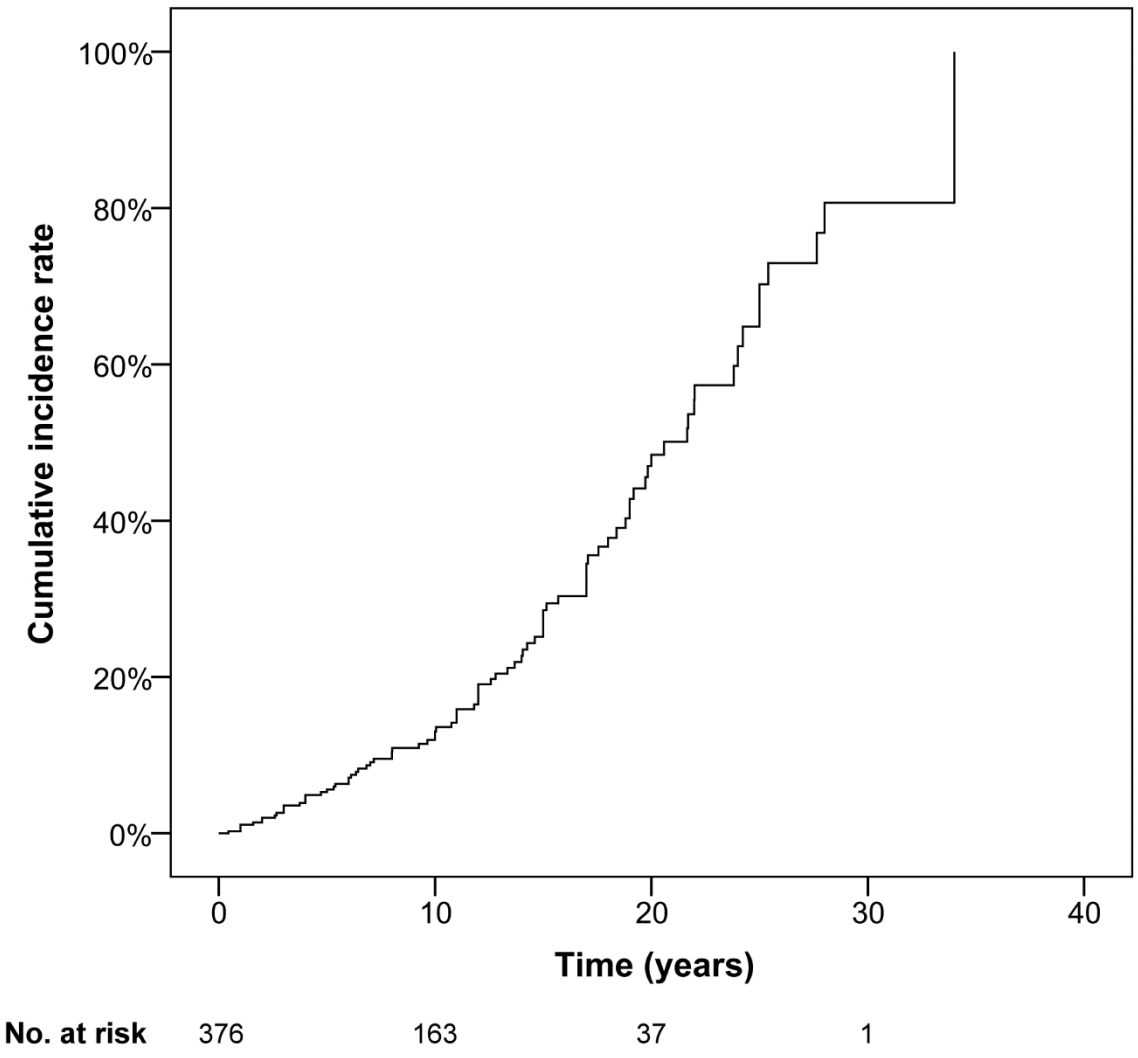
IRR: incidence rate ratio, CI: confidence interval, AK: actinic keratosis

*Estimates from Poisson regression models including terms for age and gender

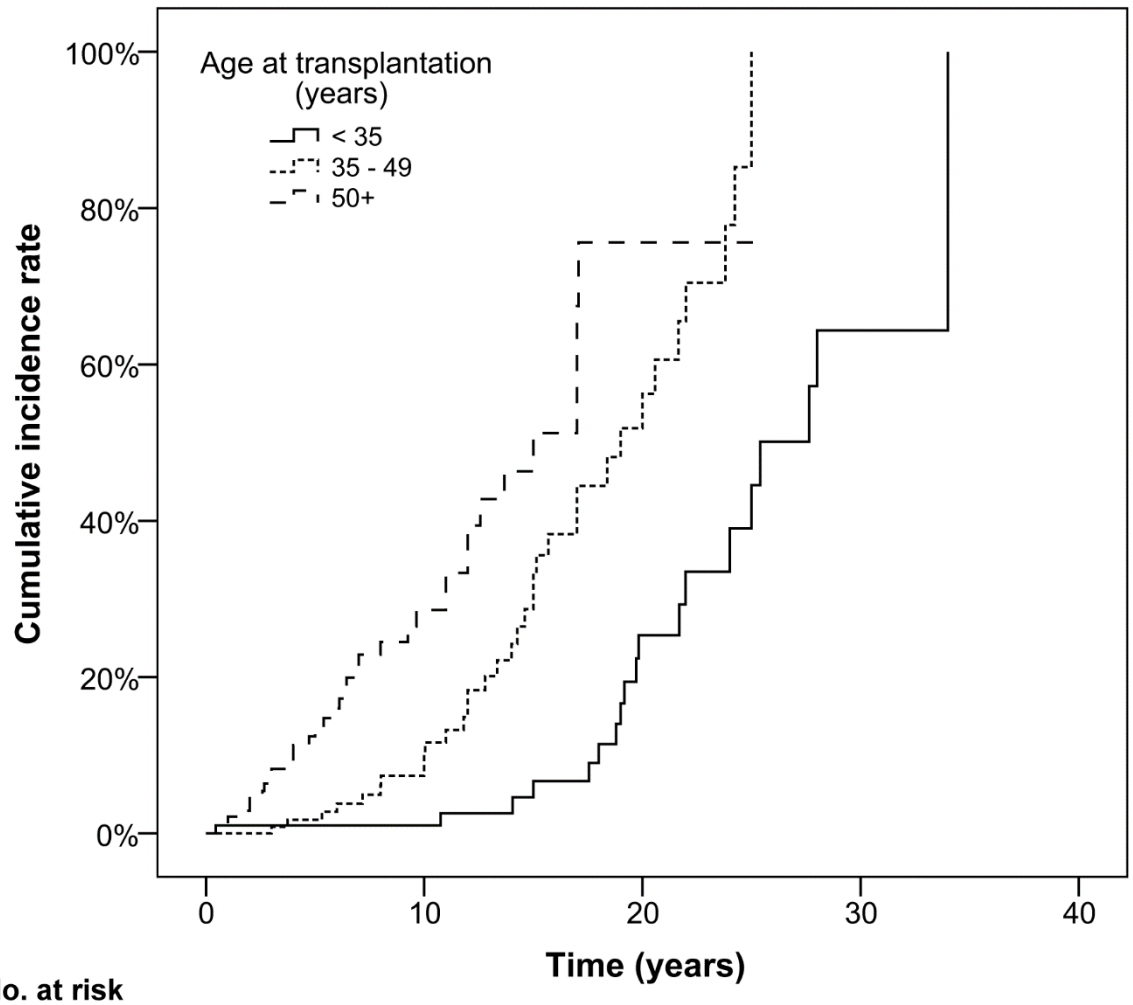
^ Estimate obtained by applying a continuity correction to univariate Poisson model

Figure 1 NMSC cumulative incidence rates since transplantation, overall (a) and by age groups (b)

a)



b)

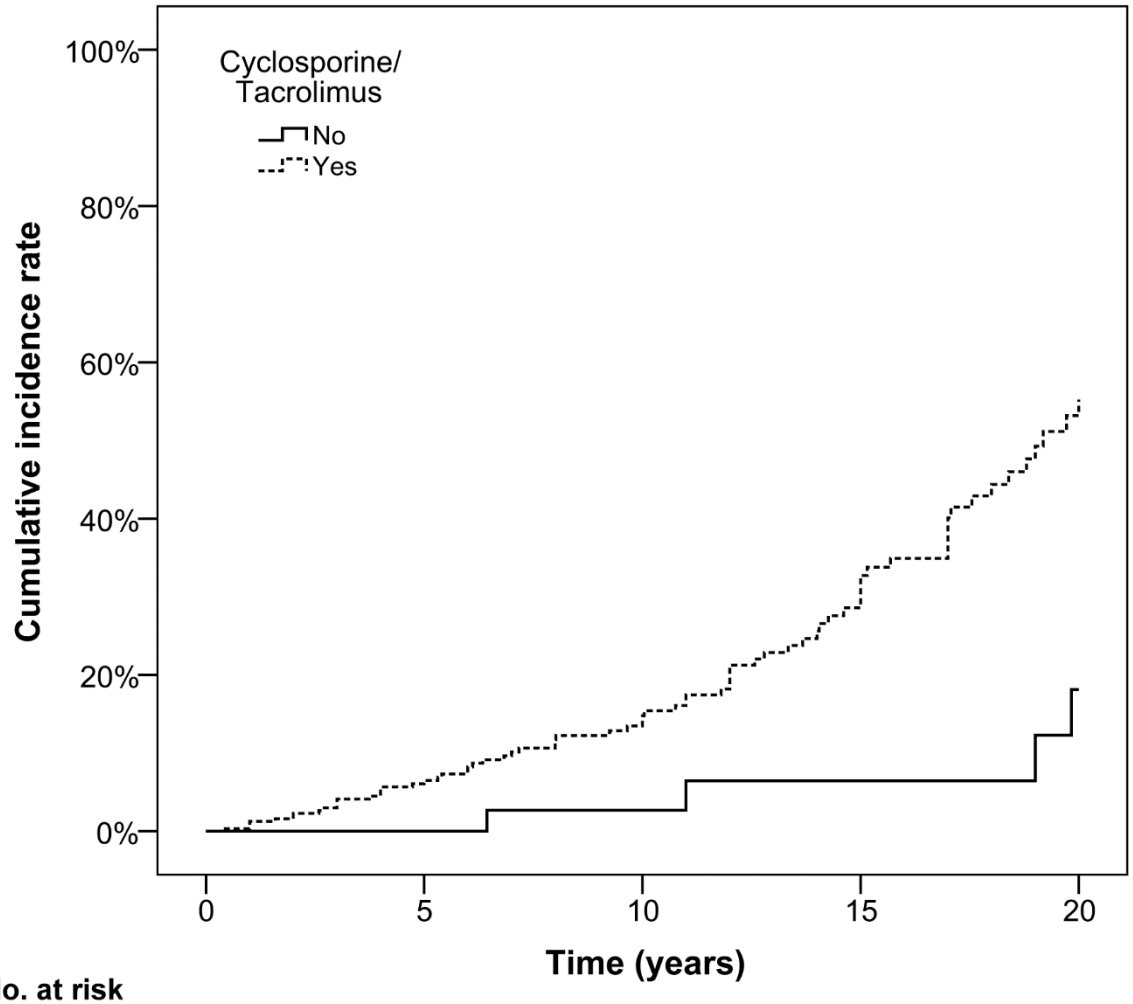


No. at risk

< 35 years	97	65	25	1
35 - 49 years	126	65	11	0
50+ years	148	33	1	0

Figure 2 NMSC cumulative incidence rates in the first 20 years after transplantation, by use of Cyclosporine/Tacrolimus (a) and Sirolimus/Everolimus (b)

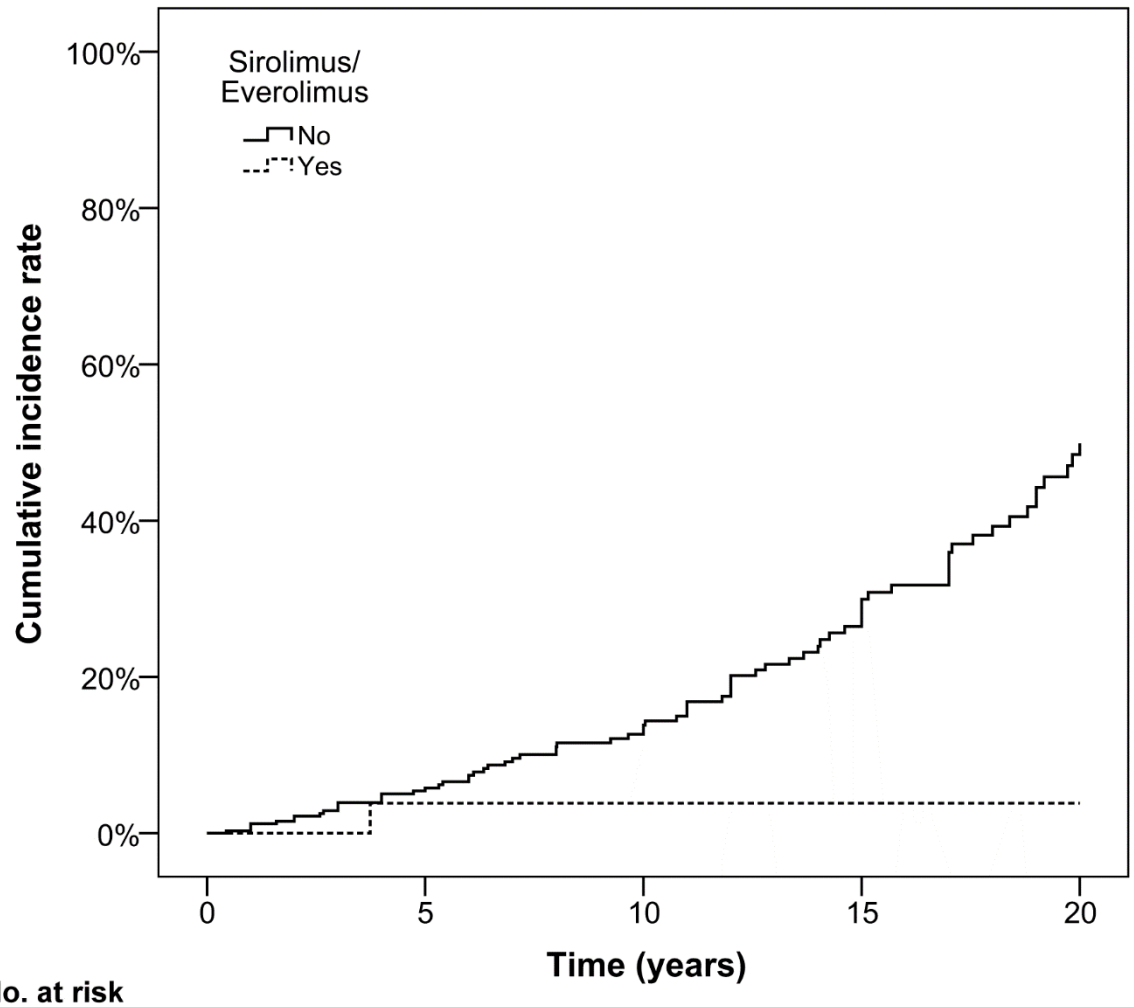
a)



No. at risk

Cyclo/Tac no	46	39	27	19	14
Cyclo/Tac yes	324	227	136	69	23

b)



No. at risk

Sirol/Ever no	338	244	152	85	36
Sirol/Ever yes	32	22	11	3	1