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Basic and clinical features of cutaneous squamous cell carcinoma in organ transplant recipients

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CHAPTER 3

The presence of Betapapillomavirus antibodies around transplantation predicts the development of keratinocyte carcinoma in organ transplant recipients: a cohort study

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Abstract

Organ transplant recipients (OTR) have an increased risk of developing keratinocyte carcinomas (KC). The aim of this study was to correlate infection with human papillomaviruses (HPV) belonging to the Beta genus (Beta-PV) at transplantation with later development of KC.

In a cohort study, sera collected between one year before and one year after transplantation of OTR transplanted between 1990 and 2006 were tested for antibody responses against the L1 capsid antigen of Beta-PV and other HPV genera (Gamma-, Mu-, Nu-, Alpha-PV) using multiplex serology. The OTR were followed for a maximum of 22 years. Cox regression models with KC, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) as outcome variables were used.

Sixty out of 445 OTR had developed KC: 14 developed only SCC, 24 only BCC and 22 both types of KC. The time-dependent hazard ratio to develop either or both types of KC, adjusted for age, sex and transplanted organ, in OTR tested Beta-PV seropositive around time of transplantation compared to Beta-PV seronegative OTR was 2.9 (95%CI 1.3-6.4). The hazard ratio for SCC was 2.9 (95%CI 0.99-8.5) and for BCC 3.1 (95%CI 1.2-8.0). There was also an association between Mu-PV seropositivity and KC, but there were no significant associations between other HPV genera tested and KC.

A positive seroresponse for Beta-PV around transplantation significantly predicted the development of KC in OTR up to 22 years later, providing additional evidence that infection with Beta-PV plays a role in KC carcinogenesis.

Introduction

Keratinocyte carcinomas (KC) are the most prevalent malignancies seen in organ transplant recipients (OTR). The incidence of squamous cell carcinoma (SCC) is 60-250 times increased compared to the general population, and for basal cell carcinomas (BCC) this is 10-40 times.[1-4] In OTR, SCC are more frequently observed than BCC with an SCC:BCC ratio of 5:1, compared to the general population where an SCC:BCC ratio of 1:4 is found.[5]

Life-long immunosuppressive therapy is the most important risk factor for developing KC in OTR. Other important risk factors include sun exposure, male gender, older age, smoking and fair skin with susceptibility to sunburn. These are similar risk factors as in the immunocompetent population.[6,7] A role of human papillomaviruses (HPV) in the development of KC has also been frequently suggested.[8-10]

HPV are small double-stranded DNA viruses that infect epithelia of skin or mucosa. Over 150 different types have been described.[11,12] HPV has been estimated to play a causative role in 5.2% of all human cancers.[13] HPV types are classified in different classes (1 to 4) by the International Agency for Research on Cancer (IARC) based on their carcinogenic potential.[14,15]

The first time that HPV infection was linked with KC was in patients with a rare autosomal recessive disease called epidermodysplasia verruciformis (EV).[16] EV patients have an increased susceptibility to widespread Beta-PV infections of the skin that progress to SCC in one-third of the patients, mainly on sun-exposed sites.[17] In the years after transplantation, OTR start to develop a clinical picture that resembles that of EV patients. Epidemiological and experimental data have advocated a potential carcinogenic role of cutaneous HPV infection in skin cancer, especially of SCC in the immunosuppressed population.[18,19]

There are different methods to detect Beta-PV infection, based on measurement of viral protein, viral DNA or serum antibody responses.[20] Serological responses are considered as the most consistent marker of biologically relevant Beta-PV infection, reflecting a past or present infection strong enough to evoke an immune response.[8,21]

Most studies investigating the association between serological responses to HPV and SCC are cross-sectional or case-control studies, were carried out in the immunocompetent population and showed an association between detection

of Beta-PV antibodies and SCC.[22-27] As far as we know, cohort studies investigating the association between serological responses to HPV and later development of a first KC were not carried out in OTR.[20] Therefore, we designed a retrospective follow-up study to establish if there is a relationship between the presence of serological responses to HPV, in particular Beta-PV, around transplantation and the development of KC (SCC and BCC) in the years after the organ transplantation.

Material and methods

Study design

Between 1966 and 2006 a total of 2136 patients were transplanted in the Leiden University Medical Center (LUMC) (Supplementary Figure 2). OTR consisted of both kidney transplant recipients (KTR) as well as simultaneous pancreas-kidney transplant recipients (SPKTR).

During the past 25 years sera were collected from a considerable number of OTR. The most extensive source of sera consisted of samples collected from 1989 for clinical care-related screening and diagnostic purposes, which were systematically stored in the department of Medical Microbiology of the LUMC. In the scope of earlier case-control studies, additional sera had been collected and stored.[28,29] From 101 patients who participated in a prospective study pre-transplant sera were retraced from the Eurotransplant serumbank.[30] Altogether, 7912 sera were available from 1269 OTR (Supplementary Figure 2). Because of practical reasons we had to limit the number of sera to be tested to a maximum of 3000, which number of sera was thought to provide enough statistical power to answer our questions.

We selected 648 representative sera from all 164 OTR who developed KC during follow-up of which at least one serum sample was available. For each of these OTR we selected between 3 and 5 control OTR without skin cancer. These patients were matched for type of transplantation (KTR or SPKTR), sex and age at transplantation, which resulted in the selection of 2112 sera from 534 OTR without skin cancer (Supplementary Figure 2). These sera had been collected between 7 years before and 42 years after the transplantation.

Since we were specifically interested in the association between seroresponses to HPV around the transplantation and the later development of KC we further

restricted our selection to OTR who had sera collected between 1 year before and 1 year after the transplantation. This resulted in a final cohort consisting of 1880 sera from 445 OTR, of whom 60 had developed KC (14 only SCC, 24 only BCC and 22 both types of skin cancer) during the 22-year follow-up period (Figure 2). The number of sera collected in these patients ranged from 1 to 11 (mean: 4.2, median: 4.0). The remaining sera will be analyzed in a separate study.
Data about development of KC were retracted from the LUMC oncologic database, the LUMC pathology database and by hand searching of the medical files. The study was approved by the medical ethical committee of the LUMC.

Serum processing and analysis

Serum samples were obtained from -20˚C freezers at the department of Medical Microbiology. They were defrosted and pipetted in 96-wells plates. Some selected serum samples were excluded from further analysis, because either they could not be found, were not properly centrifuged initially or contained too little volume (Supplementary Figure 7). The 96-wells plates were shipped on dry ice to the German Cancer Research Center (DKFZ), Heidelberg, Germany for analysis using Luminex® technology.
This multiplex serology technique is an antibody detection method based on glutathione S-transferase capture ELISA in combination with fluorescent bead technology. Positive serology cut-off points are standardized at 200 MFI (mean fluorescence intensity), targeted on antibodies against major capsid antigen L1 of the various HPV types.[31,32] Sera were tested for seven Alpha-PV (HPV2, 3, 6, 7, 13, 16, 27b), sixteen Beta-PV (HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 93, 96), eight Gamma-PV (HPV4, 48, 50, 60, 65, 95, 101, 103), two Mu-PV (HPV1, 63), and one Nu-PV (HPV41).
The OTR were considered seropositive for specific HPV genera or specific HPV types if at least one of the sera tested between 1 year before and 1 year after the transplantation had a positive antibody response against these specific HPV genera or specific HPV types.

Statistical analyses

Baseline characteristics are given in percentages and simple means calculation. Kaplan-Meier survival analyses were used in conjunction with multivariable cox proportional hazard methods to calculate corresponding hazard ratios (HR) with 95%

confidence intervals (CI) for development of KC in relation to HPV serology. The date of transplantation was used as opening date. As censoring dates we used the date of diagnosis of first KC, the date of the patient's death, the date of last follow-up, the date lost to follow-up or the date of end of study (29th October 2012). Factors considered as potential confounders were sex, type of transplantation and age at transplantation. The Cox models were adjusted for these variables. Since the variable of HPV seropositivity had a time-scope of one year before and after the transplantation (starting point) time dependent Cox regression analyses were performed. The analyses of SCC were performed regardless of the presence of BCC among the cases and controls and the analyses of BCC were performed regardless of the presence of SCC among the cases and controls.

To investigate whether there were Beta-PV types that were the driving force causing the positive association between Beta-PV seropositivity and the development of KC we formed three subgroups: a) patients who were seronegative for any Beta-PV type, b) patients who were seropositive for the specific Beta-PV type regardless of the other Beta-PV types and c) patients who were seronegative for the specific Beta-PV type regardless of the other Beta-PV types. The cumulative incidence of KC, SCC and BCC was calculated with Kaplan-Meier survival analyses. Additionally, we performed conditional step forward logistic regression to get a rough estimation which individual Beta-PV may possibly contribute to the association between seropositivity and the development of KC, SCC and/or BCC. Starting with no variables in the model all Beta-PV types, age, sex and type of organ were introduced into the model, starting with the most relevant one until the addition of new variables did not improve the model any longer.

All analyses were done with SPSS 20.0 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA).

Results

The baseline characteristics of the 445 OTR included in the study are shown in Table 1. The majority of patients were male (65%) and most patients (79%) underwent kidney transplantation. The mean age of the patients at transplantation was 47.3 years and the mean follow-up after transplantation was 11.8 year. A mean of 4.2 sera (range 1 to 17) were available from 1 year

Table 1. Baseline characteristics of the organ transplant recipients who were included in this study.

	All patients	Keratinocyte Carcinoma		Squamous cell carcinoma, irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
		+	-	+	-	+	-
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Transplantation type							
Kidney	345 (78.0)	41 (68.3)	304 (79.0)	22 (61.1)	323 (79.0)	30 (65.2)	315 (78.9)
Kidney & pancreas	100 (22.0)	19 (31.7)	81 (21.0)	14 (38.9)	86 (21.0)	16 (34.8)	84 (21.1)
Gender							
Male	291 (65.4)	40 (66.7)	251 (65.2)	28 (77.8)	263 (64.3)	29 (63.0)	262 (65.7)
Female	154 (34.6)	20 (33.3)	134 (34.8)	8 (22.2)	146 (35.7)	17 (37.0)	137 (34.3)
Age (years) at transplantation							
Mean-median (Range)	47.3-46.8 (19.9-76.4)	47.5-47.7 (19.9-68.7)	47.3-46.7 (21.2-76.4)	50.0-50.0 (31.8-68.7)	47.1-46.5 (19.9-76.4)	45.4-46.1 (19.9-64.2)	47.5-47.0 (21.2-76.4)
Number sera*							
Mean-median (Range)	4.22-4 (1-17)	5.87-7 (1-17)	3.97-4 (1-10)	6.92-8 (1-17)	3.99-4 (1-10)	5.76-6 (1-17)	4.05-4 (1-10)
Time from Tx to first KC							
Mean - median (Range)		7.7-6.9 (0.28-19.8)		9.0-8.3 (0.78-18.6)		8.0-6.6 (0.28-19.8)	

Abbreviations: KC, keratinocyte carcinoma; Tx, transplantation.* sera available and tested for HPV antibody presence between 1 year before until 1 year after transplantation.

before to 1 year after transplantation. Among the patients who developed KC, the mean time from transplantation until the first KC was 7.7 years.

Supplementary Table 1 shows the percentages of OTR who were seropositive for any HPV type tested between one year before and one year after the transplantation, for all OTR together and stratified for those with KC, SCC and BCC. Table 2 summarizes supplementary Table 1 and provides the percentages of OTR who were seropositive for at least one HPV type per HPV genus (Beta-, Gamma-, Mu-, Nu or Alpha-PV). Seroresponses against the Beta genus were more frequently observed in the patients with KC compared to patients without KC, which reached statistical significance ($p = 0.005$) (Table 2). Patients who were seropositive for at least one HPV type of a specific genus had a greater probability for being seropositive for another HPV genus (supplementary Table 2).

Table 2. Numbers and percentages of organ transplant recipients with positive antibody responses against one or more HPV types of different HPV genera*.

	All patients	Keratinocyte carcinoma		Squamous cell, carcinoma irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
		+	-	+	-	+	-
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Any Beta-PV							
Negative	119 (26.7)	7 (11.7)	112 (29.1)	4 (11.1)	115 (28.1)	5 (10.9)	114 (28.6)
Positive	326 (73.3)	53 (88.3)	273 (70.9)	32 (88.9)	294 (71.9)	41 (89.1)	285 (71.4)
Any Gamma HPV							
Negative	113 (25.4)	12 (20.0)	101 (26.2)	6 (16.7)	107 (26.2)	10 (21.7)	103 (25.8)
Positiv	332 (74.6)	48 (80.0)	284 (73.8)	30 (83.3)	302 (73.8)	36 (78.3)	296 (74.2)
Any Mu HPV							
Negative	151 (33.9)	12 (20.0)	139 (36.1)	6 (16.7)	145 (35.5)	9 (19.6)	142 (35.6)
Positive	294 (66.1)	48 (80.0)	246 (63.9)	30 (83.3)	264 (64.5)	37 (80.4)	257 (64.4)
Nu HPV							
Negative	367 (82.5)	46 (76.7)	321 (83.4)	27 (75.0)	340 (83.1)	37 (80.4)	330 (82.7)
Positive	78 (17.5)	14 (23.3)	64 (16.6)	9 (25.0)	69 (16.9)	9 (19.6)	69 (17.3)
Any Alpha-HPV							
Negative	106 (23.8)	13 (21.7)	93 (24.2)	8 (22.2)	98 (24.0)	10 (21.7)	96 (24.1)
Positive	339 (76.2)	47 (78.3)	292 (75.8)	28 (77.8)	311 (76.0)	36 (78.3)	303 (75.9)

* HPV types tested; seven Alpha- (HPV2, 3, 6, 7, 13, 16, 27b), sixteen Beta- (HPV5, 8, 9, 15, 17, 20, 23, 24, 36, 38, 49, 75, 76, 92, 93, 96), eight Gamma- (HPV4, 48, 50, 60, 65, 95, 101, 103), two Mu-types (HPV1, 63), and one Nu-type (HPV41).

The cumulative incidence of KC in Beta-PV seronegative and Beta-PV seropositive OTR is depicted in Figure 1 and supplementary Figure 1. Log rank tests were performed and showed a significant difference between Beta-PV-seropositive and seronegative OTR ($p = 0.043$). The Kaplan Meier plots for SCC and BCC showed similar curves as the plots for KC (supplementary Figures 2 and 3). Table 3 shows the time-dependent non-adjusted hazard ratios and the hazard ratios adjusted for age at transplantation, sex and type of transplantation for the development of KC, SCC and BCC. A positive association was found between seropositivity for at least one Beta-PV type detected between one year before and one year after the organ transplantation and the development of KC, SCC and/or BCC. After adjustment, also

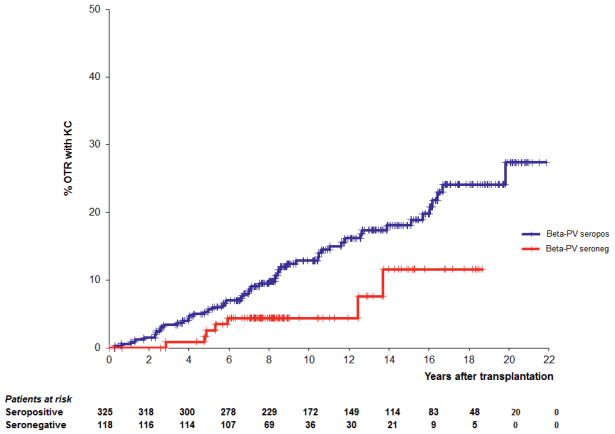

Figure 1.
Kaplan-Meier plot for development of KC in relation to HPV serology.
The cumulative incidence of keratinocyte carcinomas (KC) is statistically significantly higher in organ transplant recipients (OTR) who were seropositive against one or more HPV types of the Beta-PV genus between 1 year before and 1 year after the transplantation (p = 0.043).

Mu-PV seropositivity was associated with KC (Table 3). The Cox proportional hazard analyses (Table 3) and the Kaplan Meier plots of cumulative incidence of KC, SCC and/or BCC in HPV seronegative and seropositive OTR for the other HPV genera tested (Gamma-, Nu- and Alpha-PV) showed no significant association between seropositivity against any of these HPV genera (supplementary Figures 1, 2 and 3).
Beta-PV species 1 (specifically HPV8 and HPV20) and Beta-PV species 3 (specifically HPV75 and HPV76) were most relevant for the positive association between Beta-PV seropositivity and the development of KC (supplementary Figure 4). The potential importance of these Beta-PV types was confirmed for HPV8 and HPV75 by conditional step forward regression analyses and a statistically significant negative association was observed for HPV96 (Table 4).
Beta-PV species 1 (HPV8 and HPV20) and Beta-PV species 3 (HPV76) were also associated with the development of SCC (supplementary Figure 5), which was confirmed by conditional step forward regression analyses (Table 4). A statistically significant negative association was found for HPV24 and HPV38 (Table 4).
Beta-PV species 2 (HPV15) and species 3 (HPV75 and HPV76) were relevant for the development of BCC (supplementary Figure 6), which was confirmed in the conditional step forward regression analyses for HPV15 and HPV26, whereas there was a statistically significant negative association with HPV9 (Table 4).

Table 3. Time dependent Cox proportional Hazard ratios for keratinocyte carcinoma, squamous cell carcinoma and basal cell carcinoma in organ transplant recipients related to HPV genus serostatus between 1 year before and one year after the transplantation.

	Keratinocyte carcinoma		Squamous cell, carcinoma irrespective of basal cell carcinoma		Basal cell carcinoma, irrespective of squamous cell carcinoma	
	Unadjusted hazard ratio	**Adjusted* hazard ratio**	**Unadjusted hazard ratio**	**Adjusted* hazard ratio**	**Unadjusted hazard ratio**	**Adjusted* hazard ratio**
Beta-PV	2.2 (1.0-4.9)	2.8 (1.3-6.4)	2.1 (0.75-6.1)	2.9 (0.99-8.5)	2.3 (0.92-6.0)	3.1 (1.2-8.0)
G	1.1 (0.59-2.1)	1.3 (0.66-2.4)	1.2 (0.51-3.0)	1.7 (0.68-4.1)	0.97 (0.48-2.0)	1.1 (0.54-2.2)
Mu-PV	1.8 (0.95-3.4)	2.0 (1.0-3.7)	2.1 (0.86-5.0)	2.4 (0.98-5.7)	1.8 (0.86-3.7)	1.9 (0.93-4.0)
Nu-PV	1.2 (0.68-2.2)	1.5 (0.80-2.7)	1.3 (0.61-2.7)	1.8 (0.84-4.0)	1.0 (0.46-2.0)	1.1 (0.53-2.3)
Alpha-PV	0.93 (0.50-1.7)	0.99 (0.53-1.8)	0.84 (0.38-1.9)	0.97 (0.44-2.2)	0.92 (0.46-1.9)	0.97 (0.48-2.0)

* Adjusted for sex, age at transplantation and type of transplantation.

Discussion

We have found a statistically significant association between the development of KC, SCC and BCC after transplantation and antibody responses to one or more Beta-PV types, measured in serum drawn in a window between one year before and one year after organ transplantation. The antibody response against Beta-PV types was specific for the Beta genus since there were no significant associations for positive seroresponses against the Gamma, Nu and Alpha genera with the development of KC, SCC or BCC. Interestingly we found also a positive association between KC and Mu-PV seropositivity, an HPV genus not recognized previously as potentially associated with KC development.

So far, most studies investigating HPV seroresponses in association with KC development concerned cross-sectional and case-control studies in the immunocompetent population.[20] We are aware of only a few published cohort studies that were performed in the immunocompetent population. A Scandinavian study described a weak association for overall Beta-PV seropositivity prior to SCC diagnoses, whereas a significant association was observed for Beta-PV species 2 (OR = 1.3, 95% CI: 1.1, 1.7), and for serum samples taken more than 18 years

Table 4. Association between 16 different Beta-PV types and skin cancer.

	Keratinocyte carcinoma		Squamous cell carcinoma		Basal cell carcinoma	
	All variables in the regression model#	**Forward regression final model**	**All variables in the regression model**	**Forward regression final model**	**All variables in the regression model**	**Forward regression final model**
Species 1						
HPV5	1.3 (0.57;3.1)		1.7 (0.54;5.1)		1.2 (0.46;3.1)	
HPV8	**2.6 (1.2;5.6)**	**2.4 (1.3;4.6)**	**3.4 (1.3;8.8)**	**3.4 (1.4;8.3)**	2.2 (0.96;5.1)	
HPV20	**2.6 (1.1;5.9)**		**2.9 (1.1;8.0)**	**2.6 (1.0;6.6)**	**2.6 (1.0;6.6)**	
HPV24	0.43 (0.15;1.2)		**0.19 (0.05;0,71)**	**0.20 (0.07;0.63)**	0.68 (0.22;2.1)	
HPV36	0.50 (0.18;1.4)		0.61 (0.18;2.1)		0.40 (0.13;1.3)	
HPV93	0.48 (0.17;1.4)		0.35 (0.09;1.5)		0.27 (0.07;1.1)	
Species 2						
HPV9	**0.37 (0.13;0.99)**		0.50 (0.15;1,7)		**0.25 (0.08;0.81)**	**0.29 (0.11;0.76)**
HPV15	1.5 (0.60;3.7)		1.3 (0.38;4.2)		2.3 (0.86;6.2)	**2.7 (1.2;6.3)**
HPV17	0.82 (0.35;1.9)		0.72 (0.24)		0.95 (0.37;2.4)	
HPV23	1.3 (0.53;3.3)		**3.1 (1.0;9.8)**	2.4 (0.92;6.4)	1.4 (0.53;4.0)	
HPV38	0.94 (0.38;2.3)		**0.23 (0.06;0.83)**	**0.21 (0.07;0.66)**	0.86 (0.31;2.4)	
Species 3						
HPV49	1.4 (0.56;3.6)		1.9 (0.62;5.9)		1.4 (0.48;3.9)	
HPV75	**2.8 (1.1;7.4)**	**3.1 (1.5;6.6)**	1.6 (0.47;5.6)		2.2 (0.74;6.7)	
HPV76	1.8 (0.67;4.8)		2.3 (0.70;7.8)	**2.6 (1.1;6.5)**	1.9 (0.64;5.6)	**2.9 (1.3;6.5)**
Species 4						
HPV92	1.2 (0.49;3.1)		1.5 (0.45;4.9)		0.82 (0.28;2.4)	
Species 5						
HPV96	0.41 (0.17;1.0)	**0.38 (0.18;0.83)**	0.56 (0.19;1.7)		0.63 (0.24;1.7)	
Age*	1.0 (0.98;1.0)		1.0 (1.0;1.1)	**1.1 (1.0;1.1)**	0.99 (0.96;1.0)	
Sex*	0.87 (0.47;1.6)		1.4 (0.56;3.3)		0.81 (0.40;1.6)	
Organ*	**2.2 (1.1;4.6)**	**2.1 (1.1;4.0)**	**4.7 (1.8;12.3)**	**4.9 (2.0;12.1)**	**2.2 (1.0;4.9)**	**2.4 (1.2;4.7)**

logistic regression model
*Age: per year; *Sex: men compared to women; *Organ: combined pancreas and kidney compared to kidney. The bold numbers are statistically significant.

before diagnosis (OR = 1.8, 95% CI: 1.1, 2.8).[8] A population-based cohort study in Australia revealed no associations between the presence of any Beta-PV antibodies and the occurrence of SCC.[33] However, among people who were less

than 50 years old in 1992, the presence of Beta-PV antibodies was associated with a two-fold increased risk of SCC.[33] In the United Kingdom a prospective pilot study found no significant differences in seropositivity of any of 38 HPV types.[34] One study investigated the possible role of seropositivity for different HPVs in the incidence of a subsequent KC in 107 patients with a follow-up of 5 years who were enrolled in a previous case-control study. HPV seropositivity at baseline was strongly associated with the risk of developing a second SCC after 5 years for a number of Beta- and Gamma PV types, but no association was found for developing a second BCC.[35] As far as we know, our study is the first cohort study with a prolonged follow-up period in OTR in which the relationship between HPV serology before and just after the organ transplantation was studied in relation to later development of a first KC.

In 2011 Proby and coworkers conducted a case-control study in OTR from the Netherlands, United Kingdom, France and Italy, to investigate the association between SCC development and seroresponses against Beta-PV combined with detection of Beta-PV DNA in plucked eyebrow hairs.[10] A positive association was found for concordant DNA and seropositivity for HPV36 (OR 2.4; CI 1.0-5.4), with similar, but not statistically significant associations for HPV5, HPV9 and HPV24. Concordant Beta-PV DNA presence in hairs and a serological antibody response for at least one HPV type was significantly associated with SCC risk (OR 1.6; CI 1.1-2.5). Another case-control study conducted in immunocompetent patients in Florida U.S.A. revealed a positive association between seropositivity to increasing numbers of Beta-PV types and SCC (OR 1.9; 95% CI 1.2–3.0).[36] A similar association for Beta-PV types DNA in eyebrow hairs was found in another clinic-based case-control study (4 types vs. HPV-negative: OR 2.0, 95% CI 1.1-3.8).[37] Waterboer and colleagues performed a case-control study in an Italian immunocompetent population and found a significant two to three-fold risk of SCC with seropositivity for any species 2 Beta-PV type (OR 3.3, 95%CI 1.2-8.7) and for the Gamma-PV types (OR 3.1, 95%CI 1.1-8.6).[23] In a multi-national case-control study in immunocompetent subjects, a positive antibody response against four or more Beta-PV types was associated with a doubled risk for SCC in Australia and the Netherlands.[38]

Little data are published about the association between HPV infection and the development of BCC and the findings are contradictory. A relationship between seroresponses to Beta-PV and the development of BCC has been described

previously.[8,24,39,40] Interestingly, Paolini et al reported a high frequency (11.5%) of HPV15 DNA in a small cohort of BCC patients, which is in agreement with the observed positive association between HPV15 and BCC in our study.[41] However, some other studies did not report a positive association between Beta-PV seroreactivity and BCC.[22,25,42,43] A recent systematic review showed no association between Beta-PV serological responses and BCC.[27] In a subgroup of patients in Florida U.S.A. with Beta-PV DNA positive BCC, however, an association between a serological response against Beta-PV and BCC was observed.[43] In a population-based case-control study of patients with SCC, BCC and controls from New Hampshire U.S.A., it was found that seropositivity to Beta-PV (OR = 1.5, 95% CI = 1.0 to 2.1) was significantly associated with SCC. This risk increased with positivity for multiple HPV types. No associations were found with BCC risk.[22] Karagas et al. confirmed these findings in a larger group of 2366 skin cancer cases and controls.[25] We cannot exclude, however, that the OTR who developed BCC in our study are at increased risk of developing SCC later on, because BCC often precede the development of SCC in these patients.

Besides the advantages, the retrospective design of our study also has some disadvantages. For instance, the sera we tested were not systematically collected with the purpose to study a relationship with KC, but were randomly selected from routinely archived sera collected for HLA antibody screening and other diagnostic purposes. The hazard ratios we calculated were adjusted for sex, age at transplantation and type of transplantation.[5] We could not adjust for other risk factors for KC, like sun exposure, sunburns, skin type and type of immunosuppression, because these data were not systematically collected for all patients. These risk factors are associated with an increased prevalence of HPV DNA in eyebrow hairs, but it is not known whether this is also the case for Beta-PV seroresponses.[19,44-47]

A drawback of our study was that data about seroconversion were not systematically available. It can be speculated that seroconversion in the years following transplantation in OTR that were seronegative around transplantation, might have increased the risk of tumor development in the seronegative group, which, if true, could have led to a stronger association between Beta-PV type seropositivity and KC.

In general, a drawback of studies reporting the association between serological responses and skin cancer, is that, because of differences in the employed methodologies (initially starting with western blotting, followed by HPV-type-

specific ELISAs using L1 virus-like particles, and more recently by high throughput multiplex fluorescent bead based assays enabling the simultaneous detection of antibody responses in large series of serologic samples against a variety of HPVs) the outcomes between the different studies cannot always reliably be compared. In our study, however, this is only true for the earlier studies referred to (Western blot by Steger et al., 1990 and ELISA technique by Feltkamp et al., 2003; Stark et al., 1998), since the other studies all used the same multiplex serology technique which was performed in the same laboratory in Heidelberg.[24,39,40] Other factors that may explain the different associations between HPV infection and KC are differences in immunosuppressive regimens between centers which may exert different effects on the present HPV types, different exposure to sunlight reflected by the different latitudes of the centers and differences in exposure to different HPV types.

The exact mechanism by which Beta-PV infection predisposes for the development of KC remains unclear. It has been hypothesized that the effects of the virus on UV-induced DNA damage withholds apoptosis and DNA repair by accumulation of mutations.[48] This is supported by the fact that the E6 and E7 proteins from Beta-PV types are capable of inhibiting UV induced cell-cycle checkpoints and DNA repair mechanisms.[49-52] This potentially deregulates cell cycle control and growth in keratinocytes.[36,53] Some Beta-PV types could up-regulate the p16INK4a and Akt/P13K pathway and might play a role in the carcinogenesis of BCC.[41] The epidemiological evidence that HPV is involved in BCC carcinogenesis, however, is much weaker than the possible involvement of HPV in cutaneous SCC carcinogenesis. In conclusion, a detectable seroresponse for one or more Beta-PV types around the time of transplantation predicts an increased risk for the development of KC in OTR. This study provides additional evidence that infection with Beta-PV types plays a role in keratinocyte carcinogenesis. Additional studies will be necessary to confirm the observed association between Mu-PV infection and KC development.

Supplementary files can be found in the online version of this article

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