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Human papillomavirus and posttransplantation cutaneous squamous cell carcinoma: A multicenter, prospective cohort study

Jan N. Bouwes Bavinck¹ | Mariet C. W. Feltkamp² | Adele C. Green³ | Marta Fiocco^{4,5} | Sylvie Euvrard⁶ | Catherine A. Harwood⁷ | Shaaira Nasir⁷ | Jason Thomson⁷ | Charlotte M. Proby⁸ | Luigi Naldi⁹ | Janouk C. D. Diphooorn⁹ | Anna Venturuzzo⁹ | Gianpaolo Tessari¹⁰ | Ingo Nindl¹¹ | Francesca Sampogna¹² | Damiano Abeni¹² | Rachel E. Neale³ | Jelle J. Goeman⁴ | Koen D. Quint¹ | Anne B. Halk¹ | Carmen Sneek¹ | Roel E. Genders¹ | Maurits N. C. de Koning¹³ | Wim G. V. Quint¹³ | Ulrike Wieland¹⁴ | Sönke Weissenborn¹⁴ | Tim Waterboer¹⁵ | Michael Pawlita¹⁵ | Herbert Pfister¹⁴ | on behalf of the EPI-HPV-UV-CA group

¹Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands²Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands³QIMR Berghofer Medical Research Institute, Brisbane, Australia⁴Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands⁵Institute of Mathematics, Leiden University, Leiden, The Netherlands⁶Department of Dermatology, Edouard Herriot Hospital, Hospices Civils de Lyon, Lyon, France⁷Centre for Cell Biology and Cutaneous Research, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK⁸Division of Cancer Research, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK⁹Department of Dermatology, Azienda Ospedaliera papa Giovanni XXIII, and GISED Study Center, Bergamo, Italy¹⁰Department of Medicine, Section of Dermatology, University of Verona, c/o Ospedale Civile Maggiore, Verona, Italy¹¹Department of Dermatology, University Hospital Charité, Skin Cancer Center Charité, Berlin, Germany¹²Clinical Epidemiology Unit, IDI-IRCCS FLMM, Rome, Italy¹³DDL Diagnostic Laboratory, Rijswijk, The Netherlands¹⁴Institute of Virology, University of Cologne, Cologne, Germany¹⁵German Cancer Research Center (DKFZ), Heidelberg, Germany**Correspondence**

Jan Nico Bouwes Bavinck

Email: J.N.Bouwes_Bavinck@lumc.nl

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Organ transplant recipients (OTRs) have a 100-fold increased risk of cutaneous squamous cell carcinoma (cSCC). We prospectively evaluated the association between β genus human papillomaviruses (β PV) and keratinocyte carcinoma in OTRs. Two OTR cohorts without cSCC were assembled: cohort 1 was transplanted in 2003-2006 (n = 274) and cohort 2 was transplanted in 1986-2002 (n = 352). Participants were

Abbreviations: CI, confidence interval; BCC, basal cell carcinoma; PV, papillomavirus; cSCC, cutaneous squamous cell carcinoma; HPV, human papillomavirus; HR, hazard ratio; OTR, organ transplant recipient; UVR, ultraviolet radiation.

Members of the EPI-HPV-UV-CA group are listed in the Appendix.

[Correction added on March 13, 2018, after first online publication: authors Shaaira Nasir and Jason Thomson were added.]

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followed until death or cessation of follow-up in 2016. β PV infection was assessed in eyebrow hair by using polymerase chain reaction–based methods. β PV IgG seroresponses were determined with multiplex serology. A competing risk model with delayed entry was used to estimate cumulative incidence of histologically proven cSCC and the effect of β PV by using a multivariable Cox regression model. Results are reported as adjusted hazard ratios (HRs). OTRs with 5 or more different β PV types in eyebrow hair had 1.7 times the risk of cSCC vs OTRs with 0 to 4 different types (HR 1.7, 95% confidence interval 1.1-2.6). A similar risk was seen with high β PV loads (HR 1.8, 95% confidence interval 1.2-2.8). No significant associations were seen between serum antibodies and cSCC or between β PV and basal cell carcinoma. The diversity and load of β PV types in eyebrow hair are associated with cSCC risk in OTRs, providing evidence that β PV is associated with cSCC carcinogenesis and may present a target for future preventive strategies.

KEYWORDS

cancer/malignancy/neoplasia: risk factors, cancer/malignancy/neoplasia: skin - nonmelanoma, clinical research/practice, infection and infectious agents - viral, infection and infectious agents - viral: papillomavirus, organ transplantation in general

1 | INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC) is the most common malignancy in solid organ transplant recipients (OTRs), followed by basal cell carcinoma (BCC).^{1,2} The incidence of cSCC and BCC increases with duration of immunosuppressive therapy.¹ Important risk factors are male sex, increasing age, fair skin type, sun exposure, and smoking.³ cSCCs are usually preceded by multiple viral warts from human papillomavirus (HPV) infection and by actinic keratoses, which also often contain HPV.³

The virome component of the human microbiome is known to play an important role in disease,^{4,5} and HPV is particularly prevalent in the normal skin virome.^{6,7} HPVs are double-stranded DNA viruses that are classified into 5 genera (α , β , γ , μ , and ν).^{8,9} α HPVs (eg, mucosal HPV types 16 and 18) are responsible for the development of cervical carcinoma and other mucosal SCCs, including anogenital and oropharyngeal carcinomas.¹⁰ The role of skin β PVs in cSCC carcinogenesis is controversial,^{9,11,12} but evidence for etiologic involvement is accumulating.¹³⁻¹⁷ β PV cause latent, persistent skin infections¹⁸ and were first discovered in cSCCs from patients with epidermodysplasia verruciformis, a rare genetic skin disease characterized by increased susceptibility to β PV with a high risk of cSCC on sun-exposed skin.⁹

β PVs are ubiquitous, with more than 90% of people carrying these viruses as part of their normal skin virome.^{7,19,20} Hair follicles are the likely reservoir.¹⁸ β PV-infected cells may have impaired DNA repair and decreased sensitivity to apoptosis induced by ultraviolet radiation (UVR), facilitating escape from normal cellular defense mechanisms.^{9,13} A substantial body of epidemiologic data show an association between HPV infection and cSCC,^{12,20-24} in particular, in OTRs.^{25,26} In most studies, HPV infection has been determined by detection of serologic

responses to a large series of β PV and sometimes γ PV types^{12,21-23,25-31} and less frequently by detecting genomic DNA from 1 or multiple HPV types in eyebrow hair or skin scrapings.^{20,21,25,32-34}

Case-control studies in both OTR and immunocompetent populations have shown that the presence of β PV DNA or antibodies was associated with a 1.5- to 3-fold increased risk of cSCC.^{12,20-26,29,31,32} It was not possible, however, to determine whether the β PV infection increases risk of cSCC development or whether cSCC formation promotes active proliferation of β PV. To our best knowledge, there is only 1 published cohort study in OTRs that investigated the influence of β PV infection on the development of cSCC.²⁶ In a single-center study in 445 patients, OTRs who were β PV seropositive around the time of transplantation had an almost 3-fold increased risk of developing cSCC during the 22-year follow-up period.²⁶ There are no prospective studies in which the associations between cSCC and both the number and DNA load of β PV types have been examined.

In this study, we present data from 2 prospective multicenter cohort studies confirming that the presence of human skin β PV infection is associated with cSCC development in OTRs.

2 | METHODS

2.1 | Participants and study design

As part of a European Union–funded fifth framework program (5FP) collaborative research grant (QLK2-CT-2002-0117), the EPI-HPV-UV-CA study group collected data for a prospective³⁵ and a case-control study between 2003 and 2006.^{3,25} A description of the inclusion and exclusion criteria is provided in the supplementary material (Figures S1A-S1C). Cohort 1 was assembled from 441 OTRs

who participated in the prospective study.³⁵ Of these, 167 were excluded due to lack of sufficient follow-up data, leaving a final cohort of 274 OTRs. Cohort 2 was established from 915 OTRs who participated in the case-control study.^{3,25} Exclusion of OTRs with lack of sufficient follow-up data ($n = 242$), an cSCC diagnosed before recruitment ($n = 210$), or transplanted before 1986 ($n = 111$) gave a total of 352 OTRs. Both cohorts were under prospective observation until 2016.

Between 2003 and 2006, relevant demographic data and information about skin cancer risk factors were collected.^{3,25,35} Plucked eyebrow hair was collected; DNA was extracted for detection and genotyping of 25 β PV types using the skin β PV prototype research assay (Labo Bio-medical Products BV, Rijswijk, The Netherlands)³⁶ and for viral load determination using quantitative polymerase chain reaction.³² Because more than 95% of OTRs had 1 or more β PV types in their eyebrow hair,²⁵ we dichotomized the number of β PV as 0 to 4 and as 5 or more, as previously published.²⁵ High viral load was defined as 1 β PV copy per 1 to 19 cells and low load as 1 copy per 20 or more cells. Serum was collected and tested for 16 β PV, 6 γ PV, 4 α PV, 2 μ PV, and 1 ν PV IgG antibodies with a multiplex serology technique based on glutathione s-transferase capture ELISA in combination with fluorescent bead technology.^{37,38} All 3 techniques used have been previously described in more detail.^{25,32,35-38}

In cohort 1, eyebrow hair and serum were collected at 1, 3, 6, 9, 12, and 18 months posttransplantation. The 12-month posttransplantation time point was selected for the final analyses, because this time point best represented the β PV DNA measurement, as separate analyses have shown (Figures S2A and S2B). No participants had developed cSCC before this time point. In cohort 2, DNA and serum were collected at the time of recruitment, a median of 10 (range 2-19) years after transplantation. This was assumed to be representative of β PV DNA status in the years before sampling as β PV infection is characterized by a chronic persistent course with β PV DNA content in eyebrow hair being stable over time.¹⁸

All OTRs were routinely seen 1 to 4 times per year in specialized centers dedicated to surveillance of OTRs with skin lesions. Clinical information was collected from the time of enrolment between 2003 and 2006 until the last follow-up in 2016 and included the first histologically confirmed cSCC and BCC and the date of the last follow-up or death. We used medical charts, local pathology records, and local oncology databases to collect this information. Both cohort studies adhered to the Declaration of Helsinki principles, and the local medical ethical committees of the hospitals in the participating countries had approved the study design.

2.2 | Statistical analysis

Cohorts 1 and 2 were analyzed separately and in combination. To estimate the independent effect of risk factors on the occurrence of cSCC or BCC, a Cox model was used. Second and subsequent cSCCs or BCCs were not considered, because these data were not collected. The starting date for analysis was the day of transplantation; the end dates for those not diagnosed with cSCC or BCC were the end of follow-up or death. Proportionality of the hazard was assessed by

plotting Schoenfeld residuals for relevant covariates and by introducing interactions of relevant covariates with time in the Cox model. A value of $P < .05$ indicated a violation of the proportionality assumption.

A competing risk model with death as competing event and delayed entry (also referred to as left truncation) was used to estimate the cumulative incidence of first cSCC or BCC since transplantation.^{39,40} Competing risk models take into account situations where more than 1 cause of failure is possible. In our study, the 2 competing events were cSCC or BCC and death, as patients might have died before the occurrence of cSCC or BCC. Delayed entry was used to adjust for the fact that in cohort 2 the HPV DNA testing and serology were not performed at the time of transplantation and that we had taken the 12-month time point in cohort 1.

We had previously defined a new variable based on concordance of β PV DNA and serology. We classified OTR according to whether they were both antibody and DNA positive for the same β PV type as follows: antibody negative, regardless of DNA status; antibody positive but with no types for which DNA was also found; and antibody positive with at least 1 type concordant for DNA.²⁵

The final analyses were adjusted for sex, age, skin type, and study center (study center being an important composite variable representing differences in patient populations, immunosuppression regimens, and sun exposure between the 3 clinical sites). We also investigated the other clinical variables, and none changed the estimates of interest. These were, therefore, not included in the analysis. Analyses of cohort 2 or of the combined cohorts showed that adjustment for type of immunosuppression, type of organ transplanted, average sun exposure, number of sunburns, smoking, or alcohol consumption did not substantially change the hazard ratios (HRs) (data not shown). We did not adjust for keratotic skin lesions and common viral warts, as these potentially lie on the causal pathway between HPV infection and cSCC or BCC and their inclusion in the model may lead to overadjustment, biasing the result toward the null.⁴¹

The analyses concerning the competing risk model were performed in R environment (<http://www.R-project.org>) with the *mstate* library.⁴² All other analyses were performed with SPSS Statistics for Windows version 23.0 (IBM Corp., Armonk, NY).

3 | RESULTS

The median and maximum follow-up times since transplantation were 9.5 and 12.4 years in cohort 1 and 18.7 and 29.9 years in cohort 2, respectively. The median and maximum follow-up times since DNA and serum sampling did not differ between the cohorts and were 9.8 and 13.4 years, respectively. At the end of follow-up, 161 (26%) of 626 OTRs had died: 52 (19%) of 274 OTRs in cohort 1 and 109 (31%) of 352 OTRs in cohort 2.

The clinical profile of transplanted patients differed between cohorts 1 and 2 (Table 1), but there were no significant differences in virologic results and association with cSCC. OTRs in cohort 1 were more often female, had a darker skin type, were less often smokers, and had lower alcohol consumption. Almost all received mycophenolate

TABLE 1 Baseline characteristics of the 626 organ transplant recipients

	Cohort 1	Cohort 2	Combined cohort
No. of patients	274	352	626
Years of first transplantation	2003-2006	1986-2002	1986-2006
Study center, n (%)			
Leiden (The Netherlands)	101 (36.9)	91 (25.9)	192 (30.7)
Lyon (France)	99 (36.1)	64 (18.2)	163 (26.0)
London (UK)	32 (11.7)	123 (34.9)	155 (24.8)
Bergamo (Italy)	42 (15.3)	74 (21.0)	116 (18.5)
			P < .001
Sex, n (%)			
Women	98 (35.8)	96 (27.3)	194 (31.0)
Men	176 (64.2)	256 (72.7)	432 (69.0)
			P = .023
Age at transplantation, y			
Median	56.4	50.3	52.4
IQR (25%-75%)	47.9-62.6	38.4-57.3	42.0-58.9
			P = .003
Age at physical examination, y			
Median	56.4	59.8	58.2
IQR (25%-75%)	48.0-62.6	52.7-67.1	51.3-65.7
			P < .001
Skin phototype, n (%)			
Dark/olive	157 (57.5)	162 (46.0)	319 (51.1)
Medium	91 (33.3)	128 (36.4)	219 (35.0)
Fair	25 (9.2)	62 (17.6)	87 (13.9)
Missing values	(1)		
			P = .002
Type of organ, n (%)			
Kidney	220 (80.3)	288 (81.9)	508 (81.2)
Kidney and pancreas	34 (12.4)	29 (8.2)	63 (10.0)
Heart	20 (7.3)	35 (9.9)	55 (8.8)
			P = .140
Immunosuppressive therapy, n (%)			
Aza in any combination	14 (5.1)	148 (42.0)	162 (25.9)
MMF in any combination	251 (92.0)	108 (30.7)	359 (57.5)
CyA or Tac without Aza or MMF	8 (2.9)	96 (27.3)	104 (16.6)
Missing values	(1)		
			P < .001
Average daily sun exposure, n (%)			
1-3 h	175 (63.9)	208 (59.1)	383 (61.2)
≥4 h	99 (36.1)	144 (40.9)	243 (38.8)
			P = .224
Sunburns before the age of 20 y, n (%)			
0	110 (40.4)	174 (49.4)	284 (45.5)
1-4	108 (39.7)	123 (34.9)	231 (37.0)
≥5	54 (19.9)	55 (15.6)	109 (17.5)
Missing values	(2)		
			P = .073

(Continues)

TABLE 1 (Continued)

	Cohort 1	Cohort 2	Combined cohort
Smoking, n (%)			
No	139 (50.8)	133 (38.1)	272 (43.7)
1-19 pack-y	76 (27.7)	136 (39.0)	212 (34.0)
≥20 pack-y	59 (21.5)	80 (22.9)	139 (22.3)
Missing values		(3)	P = .003
Alcohol consumption, n (%)			
No	112 (57.1)	98 (27.8)	210 (38.3)
1-19 g/d	64 (32.7)	188 (53.4)	252 (56.0)
≥20 g/d	20 (10.2)	66 (18.8)	86 (15.7)
Missing values	(78)		P < .001
Keratotic skin lesions, n (%)			
0	205 (74.8)	111 (31.6)	316 (50.6)
1-49	65 (23.7)	208 (59.3)	273 (43.6)
≥50	4 (1.5)	32 (9.1)	36 (5.8)
Missing values		(1)	P < .001
Common viral warts, n (%)			
0	234 (85.7)	203 (57.8)	437 (70.0)
1-49	33 (12.1)	103 (29.3)	136 (21.8)
≥50	6 (2.2)	45 (12.8)	51 (8.2)
Missing values	(1)	(1)	P < .001
No. of βPV types in hair			
Median	4.5	4.0	4.0
IQR (25%-75%)	2.3-6.8	2.0-8.0	2.0-7.0
			P = .643
No. of βPV types in hair			
0-4	142 (51.8)	174 (49.4)	316 (50.5)
≥5	132 (48.2)	178 (50.6)	310 (49.5)
			P = .552
βPV load (based on 8 βPV types)			
Low load (1 copy per ≥20 cells)	195 (71.2)	252 (71.6)	447 (71.4)
High load (1 copy per <20 cells)	79 (28.8)	100 (28.4)	179 (28.6)
			P = .907
HPV seropositivity			
αPV	165 (60.2)	188 (53.4)	353 (56.4)
βPV	137 (50.0)	176 (50.0)	313 (50.0)
γPV	133 (48.5)	165 (46.9)	298 (47.6)
μPV	91 (33.2)	100 (28.4)	192 (30.5)
νPV	31 (11.3)	31 (8.8)	62 (9.9)
			P > .05

The *P*-values refer to the differences between cohorts 1 and 2 and are calculated with a χ^2 test (ordinal data) or ANOVA (continuous data). Significant *P*-values are indicated in bold.

Aza, azathioprine; CyA, cyclosporin A; MMF, mycophenolate mofetil; Tac, tacrolimus.

mofetil in combination with tacrolimus, rather than azathioprine, which was the main immunosuppressive drug in cohort 2. The numbers of keratotic lesions and common viral warts were higher in cohort 2, consistent with their longer duration of immunosuppression (Table 1).

Table 2 shows the cause-specific HRs for prognostic factors associated with cSCC in the 2 cohorts combined. Results generated from individual cohort analyses are comparable (Tables S1 and S2). Male sex, increasing age, fair skin type, and immunosuppression with azathioprine were the strongest risk factors for cSCC. Because the

TABLE 2 Possible risk factors for the development of cutaneous squamous cell carcinoma in 626 organ transplant recipients (combined cohort)

	N (%)		Cause-specific univariate HR	Adjusted HR ^a
	No SCC	SCC		
No. of patients	536	90		
Study center, n (%)				
Leiden (The Netherlands)	172 (32.1)	20 (22.3)	1	1
Lyon (France)	144 (26.9)	19 (21.1)	1.4 (0.72-2.6)	0.93 (0.49-1.8)
London (UK)	126 (23.5)	29 (32.2)	1.8 (0.99-3.2)	1.6 (0.88-2.9)
Bergamo (Italy)	94 (17.5)	22 (24.4)	1.5 (0.83-2.8)	1.7 (0.90-3.1)
Sex, n (%)				
Women	176 (32.8)	18 (20.0)	1	1
Men	360 (67.2)	72 (80.0)	1.7 (1.01-2.7)	2.0 (1.2-3.5)
Age at transplantation per 10 y				
Median	51.9	54.8		
IQR (25%-75%)	41.9-58.8	46.8-61.8	1.8 (1.4-2.1)	2.1 (1.7-2.6)
Age at physical examination per 10 y				
Median	57.8	64.5		
IQR (25%-75%)	51.2-65.43	58.1-67.6	1.9 (1.5-2.3)	2.1 (1.7-2.7)
Skin phototype, n (%)				
Dark/olive	287 (53.6)	32 (35.6)	1	1
Medium	184 (34.4)	35 (38.9)	1.6 (0.99-2.6)	1.9 (1.2-3.2)
Fair	64 (12.0)	23 (25.5)	3.3 (1.9-5.6)	3.9 (2.2-7.0)
Missing values	(1)			
Type of organ, n (%)				
Kidney	434 (81.0)	74 (82.2)	1	
Kidney and pancreas	59 (11.0)	4 (4.4)	0.45 (0.16-1.2)	0.81 (0.28-2.4)
Heart	43 (8.0)	12 (13.4)	1.8 (0.97-3.3)	1.1 (0.53-2.2)
Immunosuppressive therapy, n (%)				
Aza in any combination	108 (20.2)	54 (60.0)	1	1
MMF in any combination	334 (62.4)	25 (27.8)	0.22 (0.13-0.38)	0.28 (0.15-0.50)
CyA or Tac without Aza or MMF	93 (17.4)	11 (12.2)	0.33 (0.17-0.64)	0.25 (0.13-0.50)
Missing values	(1)			
Average daily sun exposure, n (%)				
1-3 h	335 (62.5)	48 (53.3)	1	1
≥4 h	201 (37.5)	42 (46.7)	1.4 (0.90-2.1)	1.1 (0.71-1.7)
Sunburns before the age of 20 y, n (%)				
0	243 (45.4)	41 (46.1)	1	1
1-4	196 (36.7)	35 (39.3)	1.1 (0.68-1.7)	0.91 (0.56-1.5)
≥5	96 (17.9)	13 (14.6)	0.92 (0.49-1.7)	0.66 (0.33-1.3)
Missing values	(1)	(1)		
Smoking, n (%)				
No	240 (44.9)	32 (36.0)	1	1
1-19 pack-y	182 (34.1)	30 (33.7)	1.1 (0.69-1.9)	0.94 (0.56-1.6)
≥20 pack-y	112 (21.0)	27 (30.3)	1.9 (1.1-3.2)	1.1 (0.65-1.9)
Missing values	(2)	(1)		

(Continues)

TABLE 2 (Continued)

	N (%)		Cause-specific univariate HR	Adjusted HR ^a
	No SCC	SCC		
Alcohol consumption, n (%)				
No	190 (41.0)	20 (23.5)	1	1
1-19 g/d	207 (44.7)	45 (52.9)	1.6 (0.97-2.8)	1.4 (0.83-2.5)
≥20 g/d	66 (14.3)	20 (23.6)	2.5 (1.3-4.6)	1.8 (0.93-3.5)
Missing values	(73)	(5)		
Keratotic skin lesions, n (%)				
0	299 (55.9)	17 (18.9)	1	1
1-49	213 (39.8)	60 (66.7)	4.1 (2.3-7.1)	3.1 (1.7-5.5)
≥50	23 (4.3)	13 (14.4)	8.0 (3.8-16.6)	4.7 (2.0-10.9)
Missing values	(1)			
Common viral warts, n (%)				
0	387 (72.5)	50 (55.6)	1	1
1-49	113 (21.1)	23 (25.6)	1.3 (0.80-2.2)	1.3 (0.77-2.2)
≥50	34 (6.4)	17 (18.8)	2.3 (1.3-4.1)	2.5 (1.3-5.0)
Missing values	(2)			

All hazard ratios (HRs) are calculated using delayed entry and adjusting for competitive risk of death. Significant HRs are indicated in bold. Aza, azathioprine; CyA, cyclosporin A; MMF, mycophenolate mofetil; SCC, squamous cell carcinoma; Tac, tacrolimus.

^aThe adjusted HRs are calculated with the factor of interest and sex, age at physical examination, skin type, and study center included in the model.

majority (>80%) of both cohorts were kidney transplant recipients, we had insufficient statistical power to assess differences in risk of cSCC according to type of organ transplant received. As we have reported earlier,³ the number of keratotic skin lesions and common viral warts was strongly associated with the development of cSCC, with an adjusted HR of 4.7 (95% confidence interval [CI] 2.0-10.9) for OTRs with 50 or more lesions compared with those OTRs with fewer lesions. After adjustment, sun exposure, painful sunburns, and smoking were not associated with cSCC in the combined cohort (Table 2).

The number of βPV types in eyebrow hair, βPV DNA load, and serologic responses to HPV did not significantly differ between the 2 cohorts (Table 1). Figure 1 shows a higher overall cumulative incidence of cSCC in OTRs infected with multiple (≥5) HPV types measured 12 months posttransplantation in cohort 1 and after a median of 10 years posttransplantation in cohort 2 compared with OTR with fewer βPV types or noninfected OTRs (Figure 1A). The cumulative incidence of cSCC for the separate cohorts is provided in Figures S3A and S3B. Table 3 reports the association between presence of βPV DNA in eyebrow hair and subsequent development of cSCC. The combined adjusted HR was 1.7 (95% CI 1.1-2.6). More detailed information is provided in the Tables S3-S5.

High βPV load was also associated with a significantly higher cumulative incidence of cSCC compared with a low load or absent βPV types (Figure 1B and Table 3). In the combined cohort, the adjusted HR was 1.8 (95% CI 1.2-2.8). The βPV load risk factor in cohort 1 was probably driving the HRs in the combined analyses.

HPV seropositivity at the time of sampling was not associated with cSCC risk (Figures 1C and 1D and Table 3). The association was stronger but still not significant for concordant serologic responses (Figure 1D

and Table 3). We observed a weak association between γPV seropositivity and cSCC (Table 3) but no association with αPV, μPV, and νPV seropositivity (Tables S3-S5). We examined possible heterogeneity of βPV effects between cohorts 1 and 2, but this was not the case in the univariate and adjusted analyses for any of the variables in Table 3. We were unable to assess the influence of type of organ transplanted because our study populations were mostly kidney transplant recipients. There was no evidence of violation of the proportional hazard assumption.

Data on individual HPV types suggested that βPV of species 1, specifically HPV types 5, 8, 20, 21, and 36, are those most likely to be involved in cutaneous squamous carcinogenesis, with possible roles for HPV76 (species 3) and HPV92 (species 4) (Table S6). The increased risk of skin cancer was specific for cSCC; there were no statistically significant associations between HPV infection and the development of BCC (Table S7).

4 | DISCUSSION

In this observational hospital-based study of 2 OTR cohorts with a follow-up period after HPV sampling of longer than 10 years, we found that the diversity and load of infecting βPV types were associated with an approximately doubled risk of cSCC. Our results were robust after adjustment for multiple potential confounders. This level of increased risk is equivalent to that associated with other well-established risk factors such as skin phototype³ and points to an important role for the skin virome and specifically HPV in the pathogenesis of cSCC.

HPV constitute an important part of the normal human skin virome.^{6,7,19,20} There is strong evidence from laboratory studies to suggest

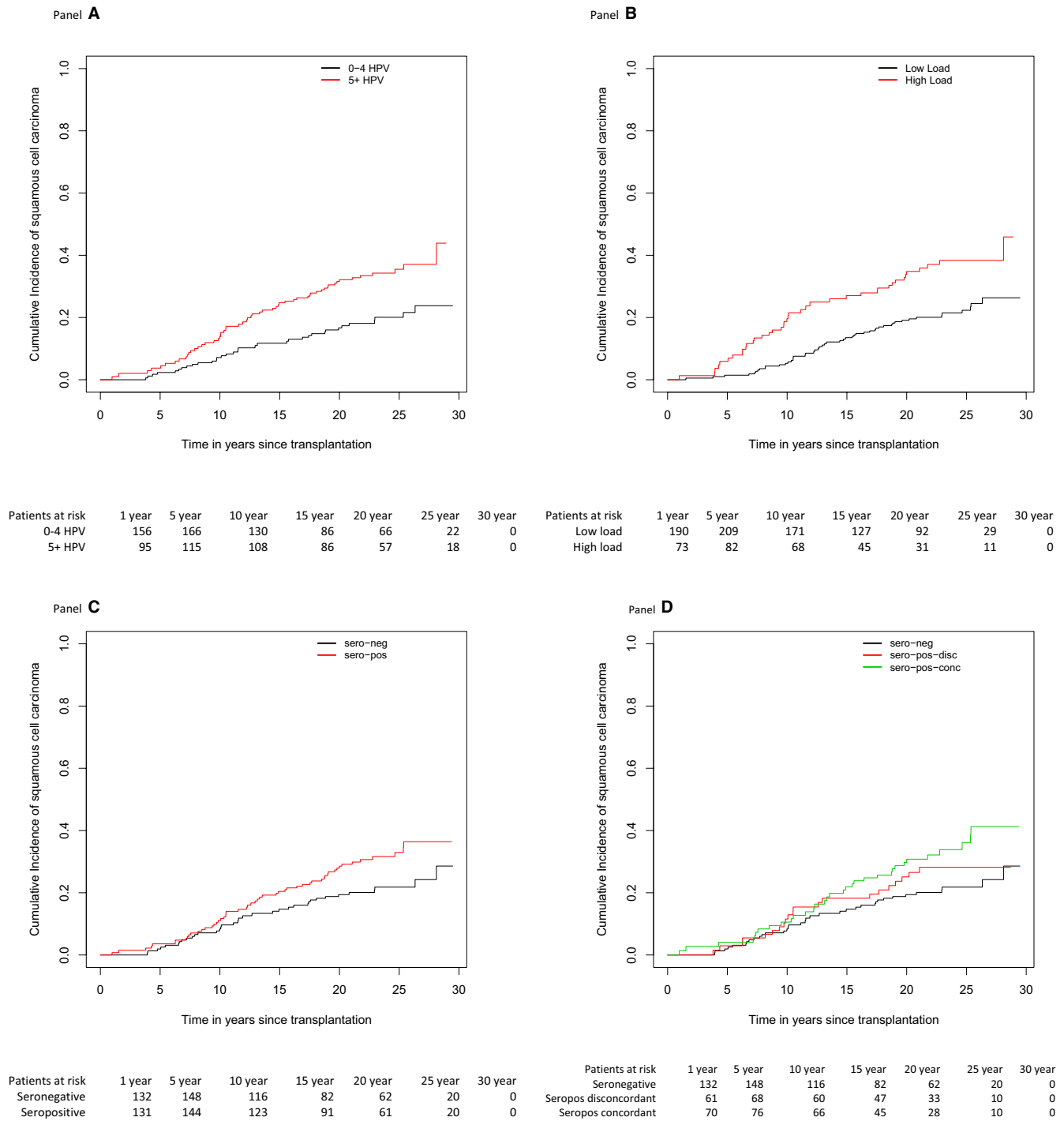


FIGURE 1 Cumulative incidence of cutaneous squamous cell carcinoma in organ-transplant recipients with 5 and more HPV types in eyebrow hair measured 12 mo post-transplant in cohort 1 and after a median of 10 years post-transplant in cohort 2 (red line) compared with transplant recipients with 0-4 HPV types (black line) (Panel A); with a high β PV load in plucked eyebrow hair (red line) compared with transplant recipients with a low load or absence of these 8 specific β PV types (black line) (Panel B); with a positive serologic response to β PV (red line) compared with transplant recipients without a positive response (black line) (Panel C); with a positive serologic response to β PV in combination with the same β PV type(s) in the eyebrow hair (green line), and with a positive serologic response to β PV in combination with different β PV types in the eyebrow hair (red line) compared with transplant recipients without a positive response (black line) (Panel D). The curves are adjusted for competitive risk of death and for delayed inclusion of the patients. Due to the delayed inclusion, the numbers of patients at risk initially increase with time before they decrease [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Risk of cutaneous squamous cell carcinoma in organ transplant recipients with human papillomavirus infection according to the presence of β PV DNA and/or serologic response to β PV

No. of squamous cell carcinoma cases/total population ^a	Cohort 1 14/274	Cohort 2 76/352	Both cohorts combined 90/626		
	Cause-specific univariate HR ^b	Cause-specific univariate HR	Adjusted HR ^c	Cause-specific univariate HR	Adjusted HR ^c
β PV DNA (based on 25 β PV types)					
0-4 types	1	1	1	1	1
≥ 5	2.6 (0.82-8.3)	1.8 (1.1-2.8)	1.6 (0.998-2.7)	1.9 (1.2-2.9)	1.7 (1.1-2.6)
β PV load (based on 8 β PV types)					
Low load (1 copy per ≥ 20 cells)	1	1	1	1	1
High load (1 copy per < 20 cells)	4.6 (1.5-13.8)	1.8 (1.1-2.8)	1.5 (0.92-2.4)	2.1 (1.4-3.1)	1.8 (1.2-2.8)
β PV serology (based on 16 β PV types)					
Seronegative	1	1	1	1	1
Seropositive	1.0 (0.36-2.9)	1.6 (1.02-2.6)	1.5 (0.90-2.4)	1.5 (0.97-2.3)	1.4 (0.90-2.1)
Combination β PV DNA and serology					
Seronegative irrespective DNA	1	1	1	1	1
Seropositive without concordant DNA	0.98 (0.25-3.9)	1.2 (0.69-2.2)	1.2 (0.66-2.2)	1.2 (0.69-2.0)	1.2 (0.71-2.1)
Seropositive with concordant DNA	1.1 (0.31-3.6)	2.0 (1.2-3.3)	1.7 (1.002-3.0)	1.8 (1.1-2.9)	1.5 (0.93-2.5)
γ PV serology (based on 6 γ PV types)					
Seronegative	1	1	1	1	1
Seropositive	1.1 (0.38-3.1)	1.5 (0.93-2.3)	1.7 (1.1-2.8)	1.4 (0.91-2.1)	1.6 (1.01-2.4)

All hazard ratios (HRs) are calculated using delayed entry and adjusting for competitive risk of death. Significant HRs are indicated in bold.

^aThe detailed numbers of squamous cell carcinoma per cohort and per exposure are provided in the supplementary material (Tables S3-S5).

^bAdjusted HRs are not available for cohort 1 because of low number of events.

^cThe HRs are adjusted for sex, age at physical examination, skin type, and study center.

that HPV plays a role in cSCC carcinogenesis, principally through synergy with UVR.¹³⁻¹⁵ In normal cells, UVR upregulates cellular defense processes leading to p53 activation, cell cycle arrest, apoptosis, or DNA repair. Several β PV types (eg, HPV5, HPV8, HPV38, and HPV49) deregulate these crucial cellular regulatory pathways by targeting multiple transcription factors and/or transcriptional regulators, resulting in infected cells that are highly susceptible to chromosomal instability and malignant transformation by UVR.^{9,15,43} Synergy between β PV and UVR has also been documented in animal models. In transgenic mice expressing the HPV8 early region or E6 gene alone, a single dose of UVR rapidly promoted papillomas and cSCC formation,¹⁶ and in HPV38 E6/E7 transgenic mice, UVR also induced the development of actinic keratoses and cSCC.¹⁷ Certain β PV types also appear to have enhanced replication in the context of host immunosuppression, likely accompanied by increased oncogene expression and oncogenic activity.⁴⁴

Previous epidemiologic studies investigating the association between β PV and cutaneous squamous carcinogenesis have generated inconsistent findings.^{9,11,12} One of the challenges is the diversity of recognized HPV types within the normal skin virome.^{33,34} More than 200 HPV types have been identified, and the number of putative new types is increasing.^{33,34,45} We tested for only a subset of HPV types. Our polymerase chain reaction-based β PV DNA detection technique was state-of-the-art when we initiated this study and remains a reliable and reproducible

method. It is possible, however, that the true association between β PV infection and cSCC is even stronger, as we may have failed to detect additional relevant HPV infections. We also observed that seropositivity to γ PV was associated with subsequent cSCC, supporting recent findings that γ PV type HPV197 is commonly present in skin tumors³⁴ and that its E6 and E7 proteins interact with a set of cellular proteins similar to those encoded by genital HPVs linked to human carcinogenesis.⁴⁵

Another limitation of this study was the lack of follow-up data in Berlin and Verona. While this decreased the power of the study, we believe that it is unlikely to have introduced bias because all patients in these 2 centers were excluded, independent of their medical history. Although cohort 1 was ideally designed for analysis of the involvement of HPV in cSCC development, we were hampered by the low number of events during the first 10 years after transplantation. Therefore, cohorts 1 and 2 were combined, which in the crude associations between the presence, number, and load of β PV DNA with the later development of cSCC appeared similar. There were also differences between the 2 cohorts. In recent years, older patients were more frequently transplanted, the immunosuppressive regimen changed, and a larger proportion of patients with a darker skin phototype were transplanted, especially in Leiden and London.

Other potential limitations of this study are missing data in the clinical characteristics included in the multivariable analyses. Of

particular concern is the 28% missing data for alcohol consumption, but we found no evidence that adjusting for alcohol in those with complete information made any difference to the estimates of association between HPV and cSCC. We have no good explanation why we did not find a statistically significant association with β PV serology. We have shown a 40% increased risk of cSCC overall in seropositive OTRs but had to exclude a substantial number of patients because of missing data, decreasing the power of our study, which may be a possible explanation of our failure to show a statistically significant association between β PV serology and cSCC.

This cohort study provides evidence that infection with β PV is associated with the development of cSCC. β PV vaccines are now in development, and our data provide a rationale for routine pretransplantation screening of the skin virome for β PV content and β PV vaccination of high-risk individuals as a possible future strategy for reducing the burden of posttransplantation cSCC.

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AUTHOR CONTRIBUTIONS

Conception and design: Jan Nico Bouwes Bavinck, Mariet C. W. Feltkamp, Adele C. Green, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Ingo Nindl, Francesca Sampogna, Damiano Abeni, Rachel E Neale, Maurits N.C. de Koning, Wim G.V. Quint, Ulrike Wieland, Tim Waterboer, Michael Pawlita, and Herbert Pfister. Collection of data: Jan Nico Bouwes Bavinck, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Janouk C.D. Diphooorn, Anna Venturuzzo, Gianpaolo Tessari, Anne Berthe Halk, and Carmen Sneek. Data analyses and interpretation: Marta Fiocco, Jelle J. Goeman, Jan Nico Bouwes Bavinck, Mariet C.W. Feltkamp, Adele C. Green, Sylvie Euvrard, Catherine A. Harwood, Charlotte M. Proby, Luigi Naldi, Ingo Nindl, Francesca Sampogna, Damiano Abeni, Rachel E Neale, Maurits N.C. de Koning, Wim G.V. Quint, Ulrike Wieland, Tim Waterboer, Michael Pawlita, and Herbert Pfister. Manuscript writing: all authors. Final approval of manuscript: all authors. Accountable for all aspects of the work: all authors.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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APPENDIX

Department of Dermatology, Leiden University Medical Center, Leiden, The Netherlands: J. N. Bouwes Bavinck, P. van der Zwan-Kralt, Y. G. L. de Graaf, L. E. Vos, E. J. Uphoff-Meijerink, A. B. Halk, C. Sneek, R. Willemze; Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands: M. C. W. Feltkamp, L. Struijk, P. Wannings, E. van der Meijden, E. I. Plasmeijer; Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands: R. Wolterbeek; Department of Dermatology, Hospices Civils de Lyon, Lyon, France: S. Euvrard, A. C. M. A. Ocampo, J. Kanitakis; Department of Dermatology, University Hospital Charité, Skin Cancer Center Charité, Berlin, Germany: I. Nindl, E. Stockfleth, T. Forschner; Department of Dermatology, Azienda Ospedaliera papa Giovanni XXIII, and GISED Study Center Bergamo, Italy: L. Naldi, A. Pizzagalli, F. Sassi, E. Gotti, R. Fiocchi, J. C. D. Diphooorn; Department of Biomedical and Surgical Sciences, Section of Dermatology, University of Verona, c/o Ospedale Civile Maggiore, Verona, Italy: G. Tessari; Centre for Cutaneous Research, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK: C. A. Harwood, C. M. Proby, J. Breuer, L. Mitchell, K. Purdie, S. R. Lambert, H. Ran; Institute of Virology, University of Cologne, Cologne, Germany: H. Pfister, U. Wieland, S. Weissenborn; German Cancer Research Center (DKFZ), Heidelberg, Germany: M. Pawlita, T. Waterboer, P. Sehr, K. M. Michael; DDL Diagnostic Laboratory, Rijswijk, The Netherlands: W. G. V. Quint, M. N. C. de Koning*, J. ter Schegget*, B. Kleter, L. J. van Doorn. *Also employed by Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands; Clinical Epidemiology Unit, IDI-IRCCS, Rome, Italy: D. Abeni, F. Sampogna, S. Simoni, G. P. Petasecca Donati, C. Masini; Queensland Institute of Medical Research, Brisbane, Australia: A. C. Green, R. E. Neale, C. Olsen, P. O'Rourke; James Cook University; S. Harrison, P. Buttner.