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Longitudinal study of seroprevalence and serostability of 34 human papillomavirus types in European organ transplant recipients

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ABSTRACT

Organ transplant recipients (OTR) are at increased risk of cutaneous squamous cell carcinoma, which may be related to reactivation of human papillomavirus (HPV) infections. Measurement of change in HPV antibodies after transplantation would help to explore this hypothesis.

We measured antibodies to 34 HPV types on up to six occasions over 18 months in 441 OTRs from five European countries.

At baseline (mean 24 days after transplantation), 80% of all OTRs were seropositive to at least one HPV type. The beta HPV genus had the highest seroprevalence (45%). For most HPV genera baseline seroprevalence peaked between 40 and 59 years old. Most OTRs retained their serostatus over time and antibody levels were stable.

Seroprevalence in immunosuppressed OTRs is stable in the 18 months immediately after transplantation. Thus there is no short-term evidence that immunosuppression leads to new or reactivated skin infection with HPV sufficient to induce antibodies.

Introduction

Papillomaviruses are small double-stranded DNA viruses that infect the epithelial cells of skin and mucosa. To date more than 150 human papillomavirus (HPV) types have been fully characterised, and are grouped into five genera based on phylogenetic DNA analysis (Bernard et al., 2010). The alpha genus contains types that infect mucosa and skin, but the majority of skin HPV types are found in the beta, gamma, mu and nu genera.

Keratinocytic skin cancer (KC) is the most commonly diagnosed type of cancer among fair-skinned populations worldwide. Squamous cell carcinoma (SCC) is the form of KC that can metastasise, and is more fatal than the more common form, basal cell carcinoma (BCC). Organ transplant recipients (OTRs) who receive immunosuppressive therapy have an increased incidence of cutaneous neoplasia compared to the general population and a reversal of the usual BCC:SCC ratio (Bouwes Bavinck et al., 2001; Nindl and Rosl, 2008; Stark et al., 1994). More than 90% of OTRs develop skin warts and up to 40% develop skin cancer within 15 years of transplantation (Birkeland et al., 1995). These patients have an up to 100-fold increased risk of SCC and 10-fold increased risk of BCC of the skin (Kiviat, 1999), a 3-fold increase in cervical cancer that is solely caused by mucosal HPV (Vajdic et al., 2006) and an approximate 4-fold increased overall cancer risk,

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compared to the general population (Adami et al., 2003). The causes of the cancers that develop in OTRs include environmental and genetic factors, but the main factor is believed to be loss of immune control of oncogenic viruses. The type of drugs used for immunosuppression, the duration of treatment and the age of the patient also influence both the incidence and the type of cancer that develops (Dantal et al., 1998).

The majority of KCs develop on sun-exposed sites of the body and solar ultraviolet (UV) radiation is the major cause (Frost and Green, 1994). Many studies have also suggested a role for HPV in the carcinogenesis of KC, particularly SCC, and some specific types of HPV, including HPV-5, HPV-8, HPV-20 and HPV-77, have been shown to be activated by UV radiation (Akgul et al., 2005; Purdie et al., 1999; Ruhland and de Villiers, 2001). UV-induced oncogenicity of the HPV proteins E6/E7 and transcriptional activity of HPVE6/E7 in KC have also been described (Dang et al., 2006; Michel et al., 2006). Furthermore, differences in *in vitro* transforming activity potential between various cutaneous HPV types have been shown, providing further evidence for a role of specific cutaneous HPV in the development of KC (Massimi et al., 2008). Despite these findings no cutaneous high-risk HPV type with a role in KC has yet been identified.

The natural history of HPV in cervical cancer has been studied extensively, and antibodies have been identified as markers of past and present infections (Dillner, 1999). It is known that only 50% of women infected with the mucosal high-risk HPV type 16 develop antibodies to HPV-16, and this can take several months up to a year after initial infection. Once presence of HPV-16 antibodies is established, low but stable titres are detectable over at least 10 years (Dillner, 1999). In comparison, little is known about the natural history of antibodies to HPV types infecting skin. As with mucosal types, it appears that only approximately half of the people with a specific HPV type on the skin generate type-specific antibodies. The presence of antibodies reflects past or present exposure, individual immunological status and genetic susceptibility and possibly also the viral load. The presence of antibodies has been shown to be associated with cutaneous SCC (Bouwes Bavinck et al., 2010; Feltkamp et al., 2003; Karagas et al., 2006; Proby et al., 2011).

HPV antibody levels (measured as MFI) and serostatus in people who are not iatrogenically immunosuppressed are stable over time (Antonsson et al., 2010) but knowledge about what happens to antibodies to mucosal and cutaneous HPV types within the context of immunosuppression is limited. Understanding the natural history of antibodies after immunosuppression is initiated may help to further elucidate the reason for the markedly elevated risk of cutaneous SCC in people who have undergone organ transplantation.

We therefore determined the stability of antibody status of 34 HPV types among 441 OTRs from five European countries in the immediate short-term, namely, over the first 18-months post transplantation. We further analysed factors that may be associated with the presence of antibodies and with change in antibody status over time. To our knowledge, this paper describes for the first time a longitudinal study of HPV antibodies in OTRs.

Results

Participants and samples

Serum samples from 441 organ transplant recipients (OTRs) were analysed: 100 participants from Bergamo (Italy), 90 from Berlin (Germany), 101 from Leiden (the Netherlands), 100 from Lyon (France), and 50 from London (UK).

The mean age at baseline was 48 years and 65% of participants were men (Table 1). Other basic characteristics of the participants studied are presented in Table 1. The mean follow-up time was 463 days (SD 138 days) and the mean time between blood collection time points was 85 days (SD 45 days) until time point 4 (12 months) and 176 days (SD 48 days) between time points 4 and 5 (18 months). Seventy-three percent of patients ($N=320$) were followed up for a year or longer, and there was no difference in the age or sex distributions of these patients compared with all those recruited. However there was a difference in the proportion of OTRs followed up for a year or more by country with the highest proportion of follow up in the Leiden group (86%; $n=87$) and the lowest in London (48%; $n=24$). Forty-eight percent of the participants had six samples collected, 24% five samples, 10% four, 5% two and three samples, and 8% had one sample collected (these 8% were used in the baseline analyses only).

HPV seroprevalence

At the time of the first blood collection, 80% of the OTRs were seropositive to at least one of the HPV types for which we tested. The seroprevalence at baseline was highest for the beta HPV types (45%), followed by the gamma genus (44%), the mucosal alpha HPV types (33%), the mu and nu genera (32%), and was lowest for the cutaneous alpha types (23%). Type-specifically, HPV-6 and HPV-4 had the highest seroprevalences at baseline (28% and 26%, respectively) followed by HPV-1 (21%; Fig. 1). HPV-93 (4%), HPV-7 (5%) and HPV-13 (5%) (Fig. 1) had the lowest seroprevalence.

Fifty-seven of the 86 people (66%) that were seronegative at baseline kept their seronegative status over time. In the group of 29 OTRs (7%) that were seronegative at baseline but then gained seropositivity over time, we found that it was most common to seroconvert to a beta HPV type followed by, gamma, mucosal alpha, cutaneous skin and mu/nu HPV types. Eighty-seven percent of the OTRs were seropositive in at least one sample over time.

Associations at baseline between seropositivity and participant characteristics

For 25 out of 34 HPV types we found a significant association between increasing age and baseline seroprevalence, with seroprevalence peaking between 41 and 60 years and decreasing thereafter (supplementary Table 1). When we grouped the types into genera, this pattern was apparent for all genera except mu and nu and the cutaneous alpha HPV types, but was only significant for the beta HPV genus ($p=0.0005$; Table 2).

Our analysis of MFI as a continuous variable supported the above findings. Linear regression (adjusted for sex) was used to identify trends in MFI values across the different age categories. The MFI was highest in the age group 50–59 years for all HPV types, with the exception of the two mucosal alpha HPV types HPV-6 and HPV-16 (data not shown). HPV-6 had the peak of MFI in the age group 40–49 ($p<0.0001$) and HPV-16 MFI increased with age ($p=0.0003$). We found no consistent associations between sex and seropositivity (data not shown) or MFI for any HPV types.

Generally, current smokers had a higher seroprevalence for most HPV types compared to former smokers and non-smokers, although this was only significant for HPV-41 (non-smokers 7%, smokers 19% and ex-smokers 13%; $p=0.020$) (HPV type-specific data not shown). When we grouped the viruses into genera, smoking was not significantly associated with higher seropositivity for any particular HPV genus after adjustment for age, sex and centre (Table 2).

Overall, OTRs with no current warts had higher seroprevalence to most of the HPV types analysed (type-specific data not shown).

Table 1
Characteristics of study population at baseline (n=441).

Characteristic	% (*n*)						*P*-value
	All	**Bergamo**	**Berlin**	**Leiden**	**London**	**Lyon**	
n	441	100	90	101	50	100	
Sex							
Female	35.4 (156)	29.0 (29)	38.9 (35)	33.7 (34)	36.0 (18)	40.0 (40)	0.494
Male	64.6 (285)	71.0 (71)	61.0 (55)	66.3 (67)	64.0 (32)	60.0 (60)	
Age							
Age distribution							
Mean (SD)	47.6 (13.2)	47.9 (13.3)	48.3 (14.7)	47.0 (12.4)	45.9 (127)	48.2 (13.1)	
up to 30	10.9 (47)	13.0 (13)	12.2 (11)	8.9 (9)	12.0 (6)	8.0 (8)	0.355
31–40	20.6 (91)	12.0 (12)	22.2 (20)	23.8 (24)	18.0 (9)	26.0 (26)	
41–50	23.5 (104)	26.0 (26)	22.2 (20)	23.8 (24)	32.0 (16)	18.0 (18)	
51–60	24.0 (16)	27.0 (27)	17.8 (16)	28.7 (29)	20.0 (10)	24.0 (24)	
61 and older	21.0 (93)	22.0 (22)	25.6 (23)	14.8 (15)	18.0 (9)	24.0 (24)	
Organ(s) transplanted							
Kidney	87.6 (386)	100.0 (100)	98.9 (89)	82.2 (83)	100.0 (50)	64.0 (64)	< **0.0001**
Heart	4.3 (9)	0	0	0	0	19.0 (19)	
Kidney and heart	0.2 (1)	0	0	0	0	1.0 (1)	
Kidney and pancreas	7.9 (35)	0	1.1 (1)	17.8 (18)	0	16.0 (16)	
Skin phototype							
Dark/olive	48.6 (215)	70.0 (70)	6.7 (6)	56.4 (57)	34.0 (17)	65.0 (65)	< **0.0001**
Medium	35.8 (158)	29.0 (29)	54.4 (49)	38.6 (39)	42.0 (21)	20.0 (20)	
Fair	15.6 (68)	1.0 (1)	38.9 (35)	5.0 (5)	24.0 (12)	15.0 (15)	
Eye colour							
Brown	45.2 (200)	59.0 (59)	32.2 (29)	36.6 (37)	38.0 (19)	56.0 (56)	**0.0001**
Green/blue/grey	54.8 (241)	41.0 (41)	67.8 (61)	63.4 (64)	62.0 (31)	44.0 (44)	
Hair colour							
Black/brown	61.8 (273)	78.0 (78)	45.6 (41)	34.6 (35)	80.0 (40)	79.0 (79)	< **0.0001**
Dark blonde/blonde/red	38.2 (168)	22.0 (22)	54.4 (49)	65.4 (66)	20.0 (10)	21.0 (21)	
Tanning							
Tan only	48.8 (214)	67.0 (67)	6.8 (6)	58.4 (59)	34.0 (17)	65.0 (65)	< **0.0001**
Burn then tan	38.0 (167)	29.0 (29)	55.7 (49)	34.7 (35)	42.0 (21)	33.0 (33)	
Burn only	13.2 (58)	4.0 (4)	37.5 (33)	6.9 (7)	24.0 (12)	2.0 (2)	
Reaction to first bright sun at midday							
Never sunbath	19.0 (84)	36.0 (36)	0	9.9 (10)	50.0 (25)	13.0 (13)	< **0.0001**
Hardly any reaction	27.7 (122)	37.0 (37)	6.7 (6)	61.4 (62)	18.0 (9)	8.0 (8)	
A little bit of red colouring	24.9 (110)	22.0 (22)	59.4 (49)	14.8 (15)	22.0 (11)	13.0 (13)	
Sunburned	20.0 (88)	5.0 (5)	36.7 (33)	36.6 (33)	10.0 (5)	33.0 (33)	
Painfully burned	7.9 (35)	0	2.2 (2)	2.2 (2)	0	31.0 (31)	
Blistering	0.5 (2)	0	0	0	0	2.0 (2)	
Painful sunburns before 20 yrs							
Never	50.6 (221)	69.0 (69)	67.8 (61)	40.6 (41)	56.2 (27)	23.5 (23)	< **0.0001**
1–4 sunburns	34.5 (151)	26.0 (26)	26.7 (24)	41.6 (42)	29.2 (14)	45.9 (45)	
5 and more sunburns	14.9 (65)	5.0 (5)	5.6 (5)	17.8 (18)	14.6 (7)	30.6 (30)	
Smoking[a]							
Never smoked	42.2 (186)	45.0 (45)	50.0 (45)	37.6 (38)	32.6 (16)	42.0 (42)	**0.004**
Current smoker	10.2 (44)	1.0 (1)	12.2 (11)	10.9 (11)	24.5 (12)	9.0 (9)	
Ex-smoker	47.6 (210)	54.0 (54)	37.8 (34)	51.5 (52)	42.9 (21)	49.0 (49)	
Alcohol consumption[a]							
None	56.6 (225)	66.0 (66)	69.3 (61)	61.4 (62)	31.1 (14)	34.0 (22)	< **0.0001**
Low	35.1 (140)	29.0 (29)	26.1 (23)	35.6 (36)	55.6 (25)	42.0 (27)	
Medium/high	8.3 (33)	5.0 (5)	4.6 (4)	3.0 (3)	13.3 (6)	24.0 (15)	
European born							
Yes	96.6 (426)	98.0 (98)	98.9 (89)	97.0 (98)	100.0 (50)	91.0 (91)	**0.009**
No	3.4 (15)	2.0 (2)	1.1 (1)	3.0 (3)	0	9.0 (9)	
Education[a]							
School only	57.5 (253)	66.0 (66)	75.3 (67)	42.6 (43)	30.6 (16)	61.0 (61)	< **0.0001**
Tech/diploma	17.5 (77)	11.0 (11)	0	17.8 (18)	49.0 (24)	24.0 (24)	
College/university	25.0 (110)	23.0 (23)	24.7 (89)	39.6 (40)	20.4 (10)	15.0 (15)	

[a] Numbers do not sum to total due to missing data.

This association was significant for HPV-48 (no warts 14% and warts 2%; p=0.017), HPV-101 (no warts 9% and warts 0%; p=0.030) and HPV-3 (no warts 9% and warts 0%; p=0.030).

We found no other overall associations with seropositivity (Table 2) or MFI (data not shown) and other participant characteristics examined.

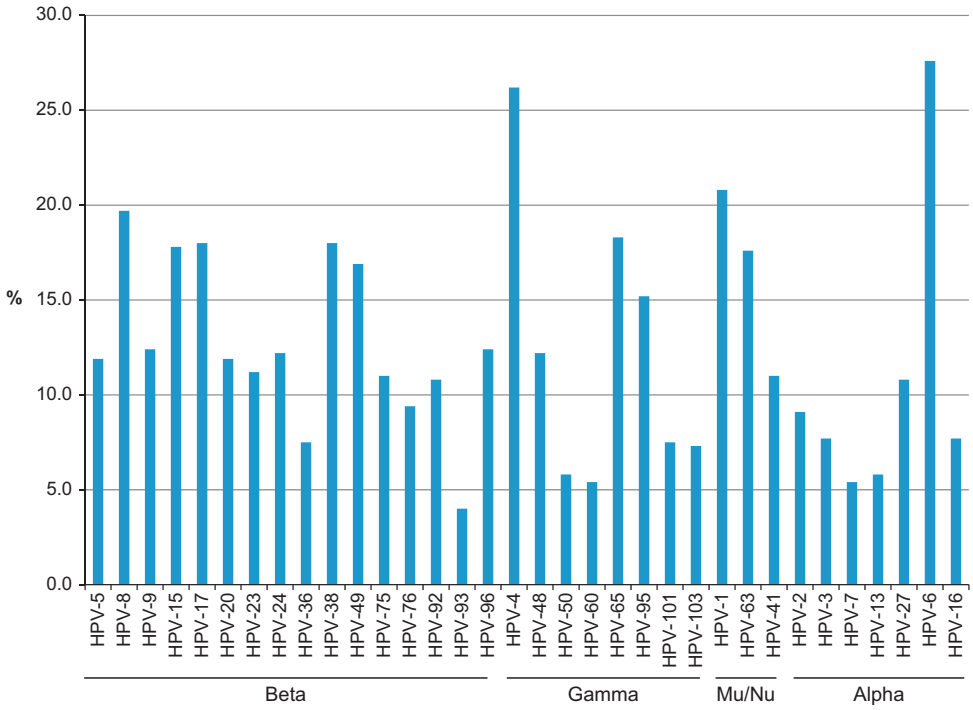

Fig. 1. Seroprevalence at baseline for the 34 HPV antibodies analysed.

Stability of HPV antibodies over time

Our longitudinal observations are based on 406 participants who had more than one sample collected. After adjustment for age, sex and centre, there was no consistent increase or decrease in seroprevalence over time for any specific HPV genus (Table 2) or type. Similarly, we found no significant trend in MFI values over time (supplementary Table 2).

We did not find a consistent increase or decrease in the number of antibody types detected. Among patients who were seropositive at baseline the median number of antibody types found was 3 at both baseline and at final follow-up. Forty percent of participants gained seropositivity to one or more HPV types, 29% had the same number of antibody types detected at baseline and at final follow-up and 31% had fewer antibody types detected at the end of the study.

Most participants were stably seronegative or seropositive over time for all HPV types (Table 3). The HPV types with the highest proportion of participants who had stable serostatus were HPV-93 (95% stable serostatus), and HPV-3, HPV-7 and HPV-60 (all three 94%). The HPV types with the highest change in serostability over time were HPV-6 (21% changed serostatus), HPV-4 (15%), and HPV-8 and HPV-65 (both 14%). Ninety percent or more of OTRs seronegative at baseline stayed seronegative over time for the majority of HPV types. OTRs seropositive at baseline were less serostable over time than those who were seronegative at baseline. For most HPV types 60% or more kept their seropositive status over time (Table 3). There was no difference in serostability for any of the HPV types in patients that were sampled over a time period of less than a year compared to patients who were followed for a year or longer.

When stratified by HPV status at baseline, we found no associations between age, sex, eye colour, hair colour, alcohol consumption, smoking, country of birth or education and serostability for any of the HPV types examined (data not shown).

Discussion

Very little is known about the natural history of HPV types or their antibodies in immunosuppressed people, particularly over time. We analysed seroprevalence and antibody stability to 34 HPV types in 441 OTRs. Eighty percent of all OTRs had at least one sample seropositive to at least one of the HPV types analysed at baseline. Previous seroprevalence studies in OTRs that are up to 15 years post transplantation have shown similar results with a range from 86% to 94% (Casabonne et al., 2009a,b,c; Proby et al., 2011). Reports in immunocompetent people have been more varied, where some studies have shown high seroprevalence (86–91%) (Antonsson et al., 2010; Casabonne et al., 2009a; Iannacone et al., 2010), and others have been considerably lower (20–60%) (Bouwes Bavinck et al., 2010; Casabonne et al., 2007; Michael et al., 2008).

We found the highest seroprevalence for the mucosal alpha type HPV-6 (28% at baseline; 26% at 18 months post transplantation). In the majority of previous studies describing HPV seroprevalence in OTRs, HPV-6 has been the most common HPV type (29–33%) (Casabonne et al., 2009a,b,c; Lally et al., 2010; Sampogna et al., 2012). Antibodies to HPV-6 are also very common in the general healthy European population, with reported seroprevalences in two different studies of 20 and 40% (Newall et al., 2008; Waterboer et al., 2009). The gamma type, HPV-4, had similarly high seroprevalence (26% at baseline). High seroprevalence to HPV-4 has commonly been reported in previous studies of both OTRs (Casabonne et al., 2009a,b,c) and immunocompetent people (Casabonne et al., 2007, 2009c; Iannacone et al., 2010; Waterboer et al., 2009). HPV-4 infection is highly prevalent in the general population, particularly in children and teenagers, as it causes common warts (Chen et al., 1993; Kilkenny et al., 1998; Pfister and zur Hausen, 1978). Overall, the HPV type-specific seroprevalences reported here in OTRs at baseline and after 18 months are similar to those previously reported for Caucasian OTRs and the general

Table 2
Associations between seropositivity, basic characteristics and time points for the different HPV genera (logistic regression adjusted for age, sex and country).

Characteristic	β HPV[a]		P-value/ trend*	γ HPV[b]		P-value/ trend*	μ/ν HPV[c]		P-value/ trend*	Skin α-HPV[d]		P-value/ trend*	Mucosal α-HPV[e]		P-value/ trend*
	%	OR (95% CI)		%	OR (95% CI)		%	OR (95% CI)		%	OR (95% CI)		%	OR (95% CI)	
Age distribution															
up to 29	27.9	1.00	**0.0005***	46.3	1.00	0.450*	38.8	1.00	0.579*	20.9	1.00	0.498*	37.8	1.00	0.120*
30–39	38.8	1.13 (1.03–1.23)		44.7	0.91 (0.75–1.12)		31.9	0.98 (0.91–1.06)		19.7	1.02 (0.95–1.10)		32.4	0.97 (0.87–1.07)	
40–49	52.0	1.22 (1.10–1.34)		50.7	0.98 (0.81–1.18)		39.9	1.04 (0.94–1.15)		20.0	1.01 (0.92–1.11)		37.8	0.98 (0.87–1.11)	
50–59	61.3	1.30 (1.17–1.44)		54.9	1.00 (0.84–1.20)		37.5	0.95 (0.85–1.06)		29.6	1.06 (0.96–1.17)		36.0	0.97 (0.86–1.09)	
60 and older	49.0	1.25 (1.11–1.40)		47.4	0.99 (0.83–1.19)		30.4	0.98 (0.86–1.10)		24.4	1.03 (0.93–1.14)		26.0	0.90 (0.80–1.01)	
Sex															
Female	49.5	1.00	0.910	54.8	1.00	0.111	40.2	1.00	0.080	27.5	1.00	0.438	38.0	1.00	0.161
Male	49.0	1.00 (0.91–1.09)		46.6	0.93 (0.85–1.02)		33.1	0.92 (0.85–1.01)		21.3	0.97 (0.90–1.05)		31.4	0.94 (0.87–1.02)	
Skin phototype															
Dark/olive	50.6	1.00	0.108	44.6	1.00	0.213	31.1	1.00	0.231	20.7	1.00	0.662	31.8	1.00	0.535
Medium	44.1	0.91 (0.83–1.00)		51.6	1.05 (0.96–1.15)		38.8	1.05 (0.95–1.15)		27.3	1.04 (0.96–1.12)		37.7	1.03 (0.95–1.13)	
Fair	56.9	1.01 (0.90–1.15)		60.9	1.11 (0.98–1.26)		43.1	1.11 (0.98–1.25)		23.4	1.02 (0.91–1.13)		30.4	0.97 (0.87–1.08)	
Eye colour															
Brown	50.0	1.00	0.263	46.4	1.00	0.465	34.3	1.00	0.593	23.2	1.00	0.830	35.1	1.00	0.583
Green/blue/grey	48.5	0.95 (0.88–1.04)		52.0	1.03 (0.95–1.12)		36.6	1.02 (0.94–1.11)		23.6	1.01 (0.94–1.09)		32.5	0.98 (0.90–1.06)	
Hair colour															
Black/brown	48.6	1.00	0.766	46.5	1.00	0.130	35.1	1.00	0.808	22.8	1.00	0.720	35.1	1.00	0.351
Dark blonde/blonde/ red	50.1	1.01 (0.93–1.10)		53.8	1.07 (0.98–1.17)		36.2	1.01 (0.93–1.10)		24.5	1.01 (0.94–1.09)		31.6	0.96 (0.89–1.04)	
Smoking															
Never	46.4	1.00	0.744	52.0	1.00	0.434	30.6	1.00	0.092	21.4	1.00	0.435	33.2	1.00	0.147
Current	49.2	1.02 (0.88–1.18)		57.1	1.06 (0.91–1.24)		40.3	1.14 (0.98–1.33)		27.2	1.08 (0.95–1.23)		42.4	1.17 (1.01–1.35)	
Ex-smoker	51.6	1.02 (0.94–1.12)		46.0	0.96 (0.88–1.06)		39.1	1.10 (1.01–1.21)		24.6	1.04 (0.96–1.13)		32.5	1.00 (0.92–1.10)	
Alcohol consumption[f]															
None	45.0	1.00	0.076	50.0	1.00	0.961	33.4	1.00	0.417	22.6	1.00	0.512	31.5	1.00	0.732
Low	58.5	1.11 (1.01–1.22)		50.2	0.99 (0.90–1.09)		39.9	1.06 (0.96–1.17)		24.8	1.04 (0.96–1.13)		33.7	1.03 (0.94–1.12)	
Medium/high	47.6	1.00 (0.85–1.19)		43.3	0.98 (0.83–1.15)		35.4	1.08 (0.91–1.27)		15.8	0.97 (0.84–1.11)		34.1	1.05 (0.90–1.23)	
European born															
Yes	48.3	1.00	0.098	49.4	1.00	0.871	43.7	1.00	0.563	23.4	1.00	0.103	43.7	1.00	0.379
No	71.2	0.99 (0.91–1.08)		48.7	0.93 (0.85–1.01)		35.2	0.92 (0.85–1.00)		12.5	0.97 (0.90–1.05)		33.3	0.94 (0.87–1.02)	
Education[f]															
School only	48.9	1.00	0.923	49.2	1.00	0.961	35.4	1.00	0.943	25.0	1.00	0.711	32.7	1.00	0.866
Tech/diploma	50.4	1.02 (0.91–1.14)		47.9	1.00 (0.88–1.12)		35.3	1.00 (0.89–1.12)		21.3	0.98 (0.84–1.09)		32.5	1.02 (0.92–1.14)	
College/university	49.1	0.99 (0.90–1.10)		51.3	0.99 (0.89–1.09)		36.3	1.02 (0.92–1.13)		21.6	0.96 (0.89–1.05)		36.8	1.02 (0.93–1.12)	
Time points[g]															
0	45.2	1.00	0.161*	44.3	1.00	0.057*	33.2	1.00	0.170*	23.0	1.00	0.941*	33.5	1.00	0.699*
1	50.1	1.05 (1.02–1.09)		49.9	1.05 (1.01–1.08)		35.2	1.03 (1.00–1.07)		24.1	1.01 (0.98–1.04)		34.6	1.02 (0.98–1.05)	
2	48.9	1.04 (1.00–1.07)		51.3	1.07 (1.03–1.11)		37.5	1.06 (1.02–1.09)		22.9	1.00 (0.97–1.03)		31.8	1.01 (0.98–1.06)	
3	50.5	1.05 (1.01–1.09)		49.2	1.04 (1.00–1.08)		35.4	1.04 (1.00–1.07)		23.1	1.01 (0.98–1.05)		33.8	1.02 (0.98–1.06)	
4	49.1	1.05 (1.01–1.09)		49.7	1.06 (1.02–1.10)		36.2	1.04 (1.01–1.07)		23.9	1.00 (0.97–1.03)		34.0	1.03 (0.99–1.07)	
5	53.0	1.06 (1.02–1.10)		54.0	1.07 (1.02–1.11)		37.5	1.04 (1.00–1.07)		24.0	1.03 (1.00–1.06)		34.5	1.04 (1.00–1.09)	

[a] β HPV types analysed; HPV-5, -8, -9, -15, -17, -20, -23, -24, -36, -38, -49, -75, -76, -92, -93, and -96.
[b] γ HPV types; HPV-4, -48,, -50, -60, -65, -95, -101, and -103.
[c] μ/ν HPV types; HPV-1, -41, and -63.
[d] Skin α HPV types; HPV-2, -3, -7, and -27.
[e] Mucosal α HPV types; HPV-6, -13, and -16.
[f] *n* does not sum up to total due to missing data.
[g] Average time between time points was 91 days.
* *P* trend.

Table 3
Serostability status for the different HPV types over time (n=406).

HPV type	% (n)							
	Seropositive at baseline				Seronegative at baseline			
	n	**Seropositive**	**Seroreversion**	**Fluctuaters**	***n***	**Seronegative**	**Seroconversion**	**Fluctuaters**
HPV-5	47	63.8 (30)	17.0 (8)	19.2 (9)	359	95.0 (341)	1.7 (6)	3.3 (12)
HPV-8	82	68.3 (56)	18.3 (15)	13.4 (11)	324	91.0 (295)	5.6 (18)	3.4 (11)
HPV-9	52	65.4 (34)	23.1 (12)	11.5 (6)	354	96.6 (342)	2.8 (10)	0.6 (2)
HPV-15	72	70.8 (51)	16.7 (12)	12.5 (9)	334	91.3 (305)	4.2 (14)	4.5 (15)
HPV-17	73	74.0 (54)	9.6 (7)	16.4 (12)	333	91.6 (305)	3.9 (13)	4.5 (15)
HPV-20	50	62.0 (31)	22.0 (11)	16.0 (8)	356	95.2 (339)	2.5 (9)	2.2 (8)
HPV-23	46	76.1 (35)	15.2 (7)	8.7 (4)	360	95.6 (344)	1.7 (6)	2.8 (10)
HPV-24	51	74.5 (38)	9.8 (5)	16.7 (8)	355	95.8 (340)	2.0 (7)	2.2 (8)
HPV-36	31	67.8 (21)	29.0 (9)	3.2 (1)	375	94.7 (355)	2.7 (10)	2.7 (10)
HPV-38	75	74.7 (56)	16.0 (12)	9.3 (7)	331	92.8 (307)	3.3 (11)	3.9 (13)
HPV-49	68	75.0 (51)	16.2 (11)	8.8 (6)	338	96.1 (325)	1.8 (6)	2.1 (7)
HPV-75	43	76.8 (33)	11.6 (5)	11.6 (5)	363	93.1 (338)	4.1 (15)	2.8 (10)
HPV-76	38	71.1 (27)	18.4 (7)	10.5 (4)	368	94.6 (348)	2.2 (8)	3.2 (12)
HPV-92	43	67.5 (29)	20.9 (9)	11.6 (5)	363	93.7 (340)	3.9 (14)	2.4 (9)
HPV-93	15	80.0 (12)	13.3 (2)	6.7 (1)	391	95.1 (372)	2.8 (11)	2.1 (8)
HPV-96	51	72.5 (37)	11.8 (6)	15.7 (8)	355	93.2 (331)	4.0 (14)	2.8 (10)
HPV-4	107	78.5 (84)	7.5 (8)	14.0 (15)	299	87.3 (261)	3.7 (11)	9.0 (27)
HPV-48	49	67.4 (33)	24.4 (11)	10.2 (5)	357	93.8 (335)	4.8 (17)	1.4 (5)
HPV-50	23	69.6 (16)	8.7 (2)	21.7 (5)	383	94.8 (363)	3.1 (12)	2.1 (8)
HPV-60	21	57.1 (12)	23.8 (5)	19.1 (4)	385	96.1 (370)	1.8 (7)	2.1 (8)
HPV-65	74	73.0 (54)	16.2 (12)	10.8 (8)	332	89.5 (297)	4.8 (16)	5.7 (19)
HPV-95	64	78.1 (50)	9.4 (6)	12.5 (8)	342	92.1 (315)	3.8 (13)	4.1 (14)
HPV-101	30	86.7 (26)	10.0 (3)	3.3 (1)	376	92.8 (349)	3.7 (14)	3.5 (13)
HPV-103	29	75.9 (22)	13.8 (4)	10.3 (3)	377	95.2 (359)	2.9 (11)	1.9 (7)
HPV-1	84	84.5 (71)	8.3 (7)	7.2 (6)	322	90.4 (291)	4.3 (14)	5.3 (17)
HPV-63	70	82.9 (58)	12.8 (9)	4.3 (3)	336	91.7 (308)	5.4 (18)	2.9 (10)
HPV-41	44	75.0 (33)	15.9 (7)	9.1 (4)	362	94.5 (342)	3.9 (14)	1.6 (6)
HPV-2	38	81.6 (31)	13.1 (5)	5.3 (2)	368	94.3 (347)	3.0 (11)	2.7 (10)
HPV-3	31	58.1 (18)	19.3 (6)	22.6 (7)	375	97.3 (365)	1.6 (6)	1.1 (4)
HPV-7	21	57.1 (12)	23.8 (5)	19.1 (4)	385	96.4 (371)	2.1 (8)	1.6 (6)
HPV-27	44	68.2 (30)	23.7 (10)	9.1 (4)	362	92.0 (333)	3.6 (13)	4.4 (16)
HPV-6	110	65.5 (72)	22.7 (25)	11.8 (13)	296	84.5 (250)	8.1 (24)	7.4 (22)
HPV-13	24	58.4 (14)	20.8 (5)	20.8 (5)	382	95.5 (365)	2.1 (8)	2.4 (9)
HPV-16	31	64.5 (20)	16.1 (5)	19.4 (6)	375	91.2 (342)	4.0 (15)	4.8 (18)

population where similar methodology has been used, and suggests that this distribution is similar worldwide and is not strongly dependent on immune status.

In terms of HPV genera, seroprevalence was highest for the beta genus and lowest for the cutaneous alpha types. However there was considerable variability in the number of types included in each genus, so the precision of seroprevalence estimates may not be directly comparable.

At baseline we found significant increasing seroprevalence with increasing age up to 59 years for most HPV types, with highest seroprevalence in the age groups between 40 and 59 years, followed by a drop in seroprevalence after 60 years. Similar results in seroprevalence, with a peak after the age of 40 have previously been reported for the beta HPV genus in an Italian general population (Michael et al., 2008; Waterboer et al., 2009). Cutaneous HPV DNA prevalence in healthy skin has been reported to increase steadily with age throughout life in immunocompetent people and OTRs with no decrease after the age of 60 (Antonsson et al., 2000; de Koning et al., 2009; Gottschling et al., 2009; Hazard et al., 2007). Since HPV infection of the skin appears to increase with age, the decrease in HPV seroprevalence after the age of 60 could possibly be due to immunosenescence and decaying antibodies in the aging population.

At baseline current smokers had an overall higher seroprevalence compared to non-smokers or former smokers but this was only significant for HPV-41. Previous studies have found significant associations with smoking and cutaneous HPV seropositivity in immunocompetent people(Andersson et al., 2008; Antonsson et al., 2010; Iannacone et al., 2010), but no association has been found in immunosuppressed OTRs (Casabonne et al., 2009a,b; Sampogna et al., 2012). It has previously been suggested that smoking could prolong the duration of mucosal alpha HPV infection (Giuliano et al., 2002) and smoking is an established cofactor in cervical carcinogenesis (Castellsague and Munoz, 2003; McIntyre-Seltman et al., 2005), but the role of smoking in cutaneous HPV infection and seroconversion is currently unknown.

Warts in the OTR population are not only caused by the HPV types that cause warts in the general population, but also by HPV types that would cause subclinical infections in immunocompetent people (Kohler et al., 2011). When we compared OTRs with and without current skin warts at baseline we found that participants with no warts had higher overall seroprevalence than participants with warts. It is possible that this is due to the generation of antibodies during the process of clearing HPV infections that cause warts, and that these antibodies protect against future viral challenges. Cell-mediated immunity that is suppressed in all solid organ and bone marrow transplant patients is believed to be the first response to HPV infection, with neutralising antibodies to the major capsid (L1) protein of HPV being produced later (Stanley, 2009). Once L1 HPV antibodies are generated they supplement the cell-mediated immune response to protect against viral challenges by that particular HPV type. In this immunosuppressed population, during the 18 month time frame studied here, we did not observe a change in antibody

production for the 34 HPV types studied here. However, production of antibodies to new antigens requires antigen presentation by the cell-mediated arm of the immune system. We cannot tell if antibody stability observed here is because there are no new viral infections or change in viral load, or if it is because cell-mediated antigen presentation to new or changed viral infections is hampered by immune suppressive treatment. Also, because we used an IgG-based sero-assay, we were unable to detect IgM responses that may have been induced in the absence of a T helper cell response.

Information about stability of cutaneous HPV antibodies over time is very limited, with only one study from a healthy population to date (Antonsson et al., 2010). We found that the cutaneous HPV antibodies studied here in OTRs were relatively stable over time, although we were unable to analyse changes from before to after transplantation. For most HPV types, 90% or more stayed either stably seropositive or seronegative over time. Generally, the cutaneous HPV types with the largest changes in serostatus were the HPV types with lower seroprevalence, and changes may be due to statistical instability rather than being a true biological effect (Antonsson et al., 2010). This is supported by the finding that we did not find any significant increasing or decreasing trends over time for MFI or seroprevalence.

We found no previous study analysing mucosal type HPV antibody stability in OTRs. Previous studies of serostability for the mucosal alpha HPV types in immunocompetent people have shown that antibody levels are usually very stable over long periods of time for the high-risk HPV types that cause cervical cancer (af Geijersstam et al., 1998; Carter et al., 1996; Dillner, 1999; Syrjanen et al., 2009), while antibody levels to the low-risk HPV types found in genital warts (e.g. HPV-6) seem to be less stable and to decay over time (Antonsson et al., 2010; Carter et al., 2000). In this study we found that all mucosal alpha HPV antibodies were very stable over time and that HPV-6 antibodies did not decay more over time than other HPV antibodies. However, people who were HPV-6 negative at baseline were more likely to seroconvert than people who were seronegative to other mucosal alpha HPV types.

A limitation of this study is the variation in time after transplantation until the first baseline sample was collected. While most baseline samples were collected within about two weeks of transplantation there was quite a broad range with one sample collected 101 days after transplantation. Although the lack of data regarding HPV serostatus prior to transplantation is a limitation as it prevents us from analysing changes from before to after immunosuppression, the half-life of IgG antibodies is approximately 26 days, so it is likely that our baseline antibody levels reflect those just prior to transplantation (Mankarious et al., 1988). Furthermore, the very stable antibody status we observed over a period when immunosuppressive regimens were likely to be changing suggests that antibody status close to the time of initiation of immunosuppression might be indicative of earlier status.

This study recruited participants in different countries in order to recruit sufficient numbers of transplant recipients. There were some demographic differences in participants from different countries, but the small numbers from each country precluded a statistically robust analysis of differences in associations or serostability across countries. However we adjusted for age, sex and country in our analyses to compensate for the demographic differences.

In conclusion, the seroprevalence and serostability to the HPV types investigated here is similar to that described for immunocompetent populations. Understanding the natural history of HPV in the context of immunosuppression is important, particularly in terms of elucidating the role of HPV in cutaneous carcinogenesis, so further research over a longer time frame or using different measures of immune function and/or HPV is warranted.

Materials and methods

Study population and data collection

Patients with recent solid organ transplants were recruited in the following hospitals: Leiden University Medical Center, Leiden, The Netherlands; Bart's and the London NHS Trust, London, UK; University Clinic Charité, Berlin, Germany; Hôpital Edouard Herriot, Lyon, France; Ospedali Riuniti di Bergamo, Bergamo, Italy; and Ospedale Civile Maggiore, Verona, Italy. The aim was to recruit 100 patients that had solid organ transplants from each centre. The recruitment of patients started in November 2002 and the last patient was recruited in January 2006. Patients were seen shortly after transplantation (mean 24 days, range 1 to 101 days after transplantation), and then on average every 3 months up to 12 months and finally at approximately 18 months. At each visit, (i) the patients had skin exams and were checked for keratotic skin lesions and skin cancer, (ii) blood and plucked eyebrow hairs were collected, and (iii) a questionnaire was filled out.

Questionnaires, together with medical charts, were used to collect information about: sex and age of the patients; type and number and date/s of solid organ transplantations; level of education; UV-related questions, such as ability to tan, sun reactivity, skin type, occupational sun exposure (during the week), recreational sun exposure during the weekends, and number of painful sunburns before the age of 20 years; and other potential risk factors for skin cancer such as smoking and alcohol consumption. Skin type (skin phototype) was re-categorized into "olive", "medium", and "fair" depending on responses to the questions about tanning ability, sun reactivity, and Fitzpatrick skin type (details described in (Bouwes Bavinck et al., 2007)). Patients with (Fitzpatrick) skin type V and VI were excluded from the study.

The study adhered to the Declaration of Helsinki Principles and the local medical ethical committees of the hospitals in the five countries had approved the study design. Participants gave their written informed consent.

HPV serology analysis

Serum samples were stored at $-80\,^{\circ}C$ and shipped on dry ice to the German Cancer Research Center (DFKZ), Heidelberg, Germany for serology analysis.

The samples were analysed for antibodies to the capsid protein (L1) of the following 34 HPV types: beta types HPV-5, -8, -9, -15, -17, -20, -23, -24, -36, -38, -49, -75, -76, -92, -93 and -96; gamma types HPV-4, -48, -50, -60, -65, -95, -101 and -103; mu types HPV-1 and -63, the nu type HPV-41, the cutaneous alpha types HPV-2, -3, -7, and -27, and the mucosal alpha types HPV-6, -13 and -16 (Bernard et al., 2010). The antibody detection method was based on glutathione S-transferase (GST) capture ELISA in combination with fluorescent bead technology (Sehr et al., 2001; Waterboer et al., 2005). Briefly, full-length viral proteins were expressed in bacteria in fusion with an N-terminal GST-domain. Glutathione cross-linked to casein was coupled to fluorescence-labeled polystyrene beads (SeroMap, Luminex, Austin, TX) and GST-fusion proteins were affinity-purified on the beads. Each fusion protein was bound to a spectrally distinct bead set, and fusion protein-loaded bead sets were mixed. Sera were incubated with the mixed bead sets at a final dilution of 1:100, and bound antibodies were detected with biotinylated goat anti-human IgG (H+L) secondary antibody and streptavidin–R-phycoerythrin. A Luminex analyser (xMAP, Luminex) was used to identify the internal colour of the individual beads and to quantify their reporter fluorescence (expressed as median fluorescence intensity (MFI) of at least 100 beads per set per serum).

Statistical analysis

We performed two different types of analyses of our HPV serology data; one where we analysed the MFI as a continuous variable and one where we dichotomised the MFI to classify people as either seropositive or seronegative to each HPV type. For this latter analysis, we also classified people according to genus seropositivity. That is, whether they were seropositive to any of the types within a particular genus. All statistical analyses were carried out using SAS (version 9.1).

A standardised cut-off was used to determine seropositivity (Michael et al., 2008).

We used linear regression to analyse associations between factors such as age, sex and smoking and MFI for each individual virus type, and logistic regression to analyse associations with seropositivity for individual virus types and for overall genera. In each case we included the MFI or serostatus at all time points in the analysis, incorporating generalised estimating equations (GEE) to take account of the intraperson correlation. Because MFI was not normally distributed, we used the log of the MFI value as the outcome variable in the linear regression analyses, and expressed the results as the ratio of the geometric means.

We conducted several analyses to examine changes in HPV serology over the 18 months following transplantation. Firstly, for each virus we classified participants as being seropositive at all time points (stably seropositive), seronegative at all time points (stably seronegative), seroconverting (changing from seronegative to seropositive over time), seroreverting (changing from seropositive to seronegative) and fluctuating between seropositive and seronegative over the available time points (fluctuating). We used simple descriptive statistics to describe the proportion of participants in each category for different viruses. Secondly, we used the logistic and linear regression models described above to analyse change in seropositivity or MFI over time, by using time point as the dependent variable in each case. These analyses were adjusted for age, sex and country of recruitment.

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Appendix A

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Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2012.10.037.

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