Merkel Cell Polyomavirus Infection in HIV-Positive Men

Ulrike Wieland, MD; Steffi Silling, PhD; Nina Scola, MD; Anja Potthoff, MD; Thilo Gambichler, MD; Norbert H. Brockmeyer, MD; Herbert Pfister, PhD; Alexander Kreuter, MD

Objective: To evaluate Merkel cell polyomavirus (MCPyV) DNA prevalence and load among men with human immunodeficiency virus (HIV) (hereafter referred to as HIV-positive men) and among healthy male control subjects.

Design: Prospective study from February 4, 2009, through April 24, 2010.

Setting: Dermatology department of a university hospital.

Patients: A total of 449 male adults were prospectively recruited, including 210 HIV-positive men who have sex with men and 239 healthy controls. Cutaneous swabs were obtained once from the surface of the forehead in all participants.

Main Outcome Measures: Swabs were evaluated for the presence of MCPyV DNA using single-round and nested polymerase chain reaction. The MCPyV DNA load (the number of MCPyV DNA copies per \( \beta \)-globin gene copy) was determined in MCPyV-positive samples using quantitative real-time polymerase chain reaction.

Results: Among 449 forehead swabs analyzed, MCPyV DNA was detected in 242 (53.9%). Compared with healthy controls, HIV-positive men more frequently had MCPyV DNA on nested polymerase chain reaction (49.4% vs 59.0%, \( P = .046 \)) and on single-round polymerase chain reaction (15.9% vs 28.1%, \( P = .002 \)). The MCPyV DNA loads in HIV-positive men were similar to those in HIV-negative men, but HIV-positive men with poorly controlled HIV infection had significantly higher MCPyV DNA loads than those who had well-controlled HIV infection (median and mean MCPyV DNA loads, 2.48 and 273.04 vs 0.48 and 11.84; \( P = .046 \)).

Conclusions: Cutaneous MCPyV prevalence is increased among HIV-positive men who have sex with men. Furthermore, MCPyV DNA loads are significantly higher in HIV-positive men with poorly controlled HIV infection compared with those who have well-controlled HIV infection. This could explain the increased risk of MCPyV-associated Merkel cell carcinoma observed among HIV-positive individuals.

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EARLY IN 2008, A NOVEL human polyomavirus, designated Merkel cell polyomavirus (MCPyV), was detected in Merkel cell carcinoma (MCC), a rare but aggressive neuroendocrine skin tumor.\(^1\) The incidence of MCC is rising steadily, with numbers almost tripling between 1986 and 2001. Approximately 1500 MCCs are diagnosed in the United States each year.\(^2,3\) Merkel cell carcinoma mainly affects older individuals of white race/ethnicity and is more common among men than among women, with a mean age at presentation of approximately 70 years.\(^4,5\) The 3-year mortality of MCC has been reported to be 33%, which is higher than that of melanoma (approximately 15%).\(^6\) Chronic exposure to UV light seems to be involved in the pathogenesis of MCC, and more than 80% of the tumors are located on sun-exposed areas of the body.\(^7\) Profound dysregulation of the immune system, such as in leukemia or in iatrogenic immunosuppression secondary to solid-organ transplantation, also seems to be a crucial risk factor for MCC.\(^3,8,9\) Moreover, an increased risk for MCC in persons with human immunodeficiency virus (HIV) infection has been reported.\(^10,11\) Since its discovery in 2008, several research groups have confirmed the high association of MCPyV with MCC; approximately 80% of MCCs demonstrate MCPyV DNA positivity.\(^1,12-20\) In MCC, integration of the viral DNA into the tumor genome is frequent.\(^1,21\) However, MCPyV was found at lower frequencies (3%-43%) in other skin tumors (eg, basal cell carcinomas, squamous cell carcinomas, and keratoacanthomas) and is frequently found in healthy skin.\(^12,19,20,22-28\) In contrast to MCC, MCPyV...
We recruited HIV-positive MSM who were older than 18 years from the infectious diseases unit of the Department of Dermatology, Venereology, and Allergology, Ruhr-University Bochum, Bochum, Germany. The US Centers for Disease Control and Prevention (CDC) classification system for HIV and AIDS (http://www.aidsetc.org/aidsetc?page=cm-105) was used to characterize the severity of HIV disease by CD4 cell counts and by the presence of specific HIV-related conditions. In all patients, current CD4 cell counts and HIV type 1 (HIV-1) viral loads were determined. Moreover, patients were interviewed regarding medical history, current use and lifetime use of highly active antiretroviral therapy (HAART), and smoking status. Patients having well-controlled HIV infection were defined as those with undetectable plasma HIV-1 RNA and CD4 cell counts exceeding 350 cells/µL. As a control group, immunocompetent HIV-negative healthy men seen at our department for skin cancer screening were included in the study (Table 1). All 449 participants underwent a complete skin examination to exclude the presence of skin cancer, skin infections, and acute and chronic inflammatory dermatoses.

For MCPyV sampling, a wooden cotton swab was forcefully rubbed over an area of about 10 cm² on the forehead of patients or controls. The protocol of the study was approved by the ethics review board of Ruhr-University of Bochum, and the study was conducted according to principles of the Declaration of Helsinki. Patients and controls gave their written informed consent to participate in the study.

**MCPyV DNA DETECTION**

DNA isolation was performed as previously described using a commercially available DNA extraction kit (QIAamp DNA Mini; Qiagen, Hilden, Germany). Real-time β-globin gene polymerase chain reaction (PCR) (LightCycler Control Kit DNA; Roche, Mannheim, Germany) was performed to demonstrate that samples contained adequate DNA and were free of substances inhibitory to PCR. For the detection of MCPyV DNA, samples were analyzed by single-round large T antigen primer set 3 PCR (309 base pair [bp]) (sPCR) and by nested MCPyV PCR (358 bp) (nPCR) as previously described, with slight modification. The LT3 and MCPyV_outer sense (OS), _outer antisense (OAS), _inner sense (IS), and _inner antisense (IAS) primer sequences have been published. Hot-start PCRs were performed in a 50-µL volume containing 5 µL of purified total cellular DNA, 30 mM potassium chloride, 20 mM Tris-HCl buffer (pH 8.4), 1.5 mM magnesium chloride, deoxynucleotide triphosphates (0.2 mM each), 2.5 U of DNA polymerase (Platinum Taq; Invitrogen, Karlsruhe, Germany), and primers (0.5 µM each). Cycling conditions (T3 Thermocycler; Biometra, Göttingen, Germany) were 94°C for 5 minutes, followed by 31 cycles of 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 60 seconds for both sPCR and nPCR. For internal nested IS/IAS PCR, 3 µL of OS/OAS PCR product was used. The PCR products (10 µL) were separated on 2% agarose gels and visualized by ethidium bromide staining. Negative control samples with water and human placental DNA instead of patient samples were included in each amplification series and never yielded positive results. The analytical sensitivity of sPCR was 1000 copies of cloned MCPyV LT3 DNA, and that of nPCR was 10 copies of cloned MCPyV LTI DNA per assay.

### METHODS

**PATIENTS AND CLINICAL EXAMINATION**

A recent pilot study performed by some of the authors of the present study found that MCPyV DNA was present in 31% of 120 anal, penile, and oral samples from men with HIV (hereafter referred to as HIV-positive men) who have sex with men (MSM); moreover, MCPyV was present in 50% (7 of 14) of plucked eyebrow hairs of HIV-positive MSM. These observations encouraged us to screen for the presence of MCPyV among a larger collective of HIV-positive MSM as well as among healthy male control subjects. Because MCC is frequently located on the head, we decided to obtain cutaneous swabs from the forehead of study participants for MCPyV DNA analysis. Given the increased risk reported in the literature for MCC development among HIV-infected individuals, we hypothesized that MCPyV would be more frequently found among HIV-positive men compared with immunocompetent male control subjects. Furthermore, we compared MCPyV DNA loads in HIV-positive and HIV-negative men and in HIV-positive men with well-controlled vs poorly controlled HIV infection.

### RESULTS

### Table 1. Characteristics of Patients and Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV-Positive MSM (n=210)</strong></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>Mean (range) 43.0 (21-75)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 43 (11)</td>
</tr>
<tr>
<td></td>
<td>Patients receiving HAART, No. (%)</td>
</tr>
<tr>
<td></td>
<td>CD4 cell count, cells/µL Mean (range) 592.6 (80-1822)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 549 (355)</td>
</tr>
<tr>
<td></td>
<td>HIV-1 RNA load, copies/mL Mean (range) 20 052 (0-884 000)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 0 (207)</td>
</tr>
<tr>
<td></td>
<td>CDC stage of HIV infection, No. (%)</td>
</tr>
<tr>
<td></td>
<td>A 52 (24.8)</td>
</tr>
<tr>
<td></td>
<td>B 92 (43.8)</td>
</tr>
<tr>
<td></td>
<td>C 60 (28.6)</td>
</tr>
<tr>
<td></td>
<td>Unknown 6 (2.9)</td>
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<tr>
<td></td>
<td>HIV infection, No. (%)</td>
</tr>
<tr>
<td></td>
<td>Well controlled 109 (51.9)</td>
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<td></td>
<td>Poorly controlled 94 (44.8)</td>
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<tr>
<td>Smoking status</td>
<td>Non-smoker 117 (55.7)</td>
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<tr>
<td></td>
<td>Current smoker 93 (44.3)</td>
</tr>
<tr>
<td><strong>Healthy Male Control Subjects (n=239)</strong></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>Mean (range) 47.0 (18-79)</td>
</tr>
<tr>
<td></td>
<td>Median (IQR) 48 (21)</td>
</tr>
<tr>
<td>Smoking status, No. (%)</td>
<td>Non-smoker 141 (59.0)</td>
</tr>
<tr>
<td></td>
<td>Current smoker 52 (21.8)</td>
</tr>
<tr>
<td></td>
<td>Unknown 46 (19.2)</td>
</tr>
</tbody>
</table>

Abbreviations: CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HIV-1, HIV type 1; IQR, interquartile range; MSM, men who have sex with men.

a Median is less than 40.
b Seven MSM who had HIV could not be classified as having controlled or uncontrolled HIV infection because of missing data.

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MCPyV DNA LOAD DETERMINATION

All samples positive on sPCR or on nPCR were analyzed by quantitative MCPyV PCR (q-PCR) using a real-time PCR platform (LightCycler 480 Real-Time PCR, Roche). The q-PCR was performed in 20 µL containing 5 µL of purified total cellular DNA and generated a 74-bp amplimer. Primer and locked nucleic acid hydrolysis probe sequences and PCR conditions have been described by Sihto et al.13 Differing from the method by Sihto et al, the elongation time at 72°C was reduced to 5 seconds. Primers were synthesized (TIB Molbiol, Berlin, Germany), and a locked nucleic acid hydrolysis probe (No. 6 of the Universal Probe Library Set, Roche) was used. Calculation of the initial MCPyV DNA copy numbers (maximum analysis, and proportional baseline adjustment) via a standard curve generated with exactly quantified MCPyV DNA plasmid standards (10-fold dilution series from 10⁶ to 10 copies) was performed using software accompanying the PCR system (LightCycler 480 [F1 channel setting, second-derivative maximum analysis, and proportional baseline adjustment]) via a standard curve generated with exactly quantified MCPyV DNA plasmid standards (10-fold dilution series from 10⁶ to 10 copies) as previously described.14 The slope of the generated standard curves indicated amplification efficiencies from 1.919 to 2.017 (mean and median, 1.962 and 1.963 [n=20]). The MCPyV DNA standard used (plasmid pJet1; Fermentas, St Leon-Roth, Germany) carried an LT3 MCPyV (309 bp) insert. Ten copies of MCPyV standard DNA were regularly (100%) detectable, and 5 copies and 1 copy were frequently (89% and 4%, respectively) detectable by q-PCR. Negative control samples (human placental DNA) were included in each run and never yielded fluorescence signals above background. The MCPyV DNA load was defined as the number of MCPyV DNA copies per β-globin gene copy. The single-copy β-globin gene was quantified using a commercially available real-time PCR kit (LightCycler Control Kit DNA).32

STATISTICAL ANALYSIS

Statistical analyses were performed using commercially available software (PASW Statistics 18.0, version 18.0.0 for Mac; SPSS Inc, Chicago, Illinois). Distribution of data was assessed by D’Agostino-Pearson test. t Test (for independent samples) was used to assess age dependence of MCPyV prevalence. Qualitative PCR data (nPCR and sPCR) were analyzed using the χ² test and Fisher exact test (2-sided). The MCPyV DNA load data (q-PCR) were analyzed using Mann-Whitney test (for 2 independent samples) or Kruskal-Wallis test (for k-independent samples). P < .05 was considered significant.

RESULTS

CHARACTERISTICS OF PATIENTS AND CONTROLS

Between February 4, 2009, and April 24, 2010, a total of 449 men (210 HIV-positive MSM and 239 HIV-negative healthy male controls) were evaluated for the presence of MCPyV DNA on the surface of the forehead skin. Characteristics of patients and controls are given in Table 1. Among HIV-positive MSM, the age range was 21 to 75 years (median age, 43 years). The median CD4 cell count was 549 cells/µL, and the median HIV-1 RNA load was less than 40 copies per milliliter. Fifty-two patients were classified as having CDC stage A disease, 92 as having stage B, and 60 as having stage C, and the CDC stage was unknown in 6 patients. One hundred seventy-one of 210 HIV-positive patients (81.4%) were receiving HAART at study enrollment. One hundred nine MSM who had HIV had well-controlled HIV infection at study enrollment.

In the latter group, almost all patients (98.2% [107 of 109]) were receiving HAART compared with 64.9% (61 of 94) in the group of patients who had poorly controlled HIV infection (P < .001) (data were unavailable for 7 patients). Ninety-three HIV-positive MSM were smokers, and 117 were nonsmokers.

In the control group, 239 HIV-negative healthy men (median age, 48 years; age range, 18-79 years) were included. Fifty-two controls were smokers, 141 were nonsmokers, and smoking status was unknown in 46 controls.

At examination, none of the HIV-positive MSM and none of the controls had cutaneous infectious diseases, skin cancer precursor lesions (eg, actinic keratosis or Bowen disease), acute or chronic inflammatory dermatoses (eg, psoriasis or atopic dermatitis), or clinical signs of skin cancer (eg, squamous cell carcinoma, basal cell carcinoma, melanoma, or MCC). None of the HIV-positive MSM and none of the control subjects had a history of skin cancer, including MCC.

Table 2. Merkel Cell Polyomavirus (MCPyV) DNA Prevalence Among HIV-Positive MSM and Among Healthy Male Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>nPCR (%)</th>
<th>sPCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-positive MSM</td>
<td>210</td>
<td>124 (59.0)</td>
<td>59 (28.1)</td>
</tr>
<tr>
<td>Well-controlled HIV infection</td>
<td>109</td>
<td>65 (59.6)</td>
<td>24 (22.0)</td>
</tr>
<tr>
<td>Poorly controlled HIV infection</td>
<td>94</td>
<td>58 (61.7)</td>
<td>35 (37.2)</td>
</tr>
<tr>
<td>Healthy male control subjects</td>
<td>239</td>
<td>118 (49.4)</td>
<td>38 (15.9)</td>
</tr>
<tr>
<td>Total</td>
<td>449</td>
<td>242 (53.9)</td>
<td>97 (21.6)</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; MSM, men who have sex with men; nPCR, nested polymerase chain reaction; sPCR, single-round polymerase chain reaction.

Overall, 53.9% and 21.6% of 449 study participants demonstrated MCPyV DNA positivity on nPCR and sPCR, respectively (Table 2). All samples positive on sPCR were also positive on nPCR. The MCPyV DNA prevalence did not vary significantly with age (P = .002). When comparing healthy male controls, HIV-positive MSM demonstrated MCPyV DNA positivity on their forehead significantly more often on nPCR (49.4% vs 59.0%, P = .046) and on sPCR (15.9% vs 28.1%, P = .002). When comparing HIV-positive men who had well-controlled HIV infection with those who had poorly controlled HIV infection, the latter group showed MCPyV DNA positivity significantly more often on sPCR (22.0% vs 37.2%, P = .02) but not on nPCR (59.6% vs 61.7%, P = .78). MCPyV prevalence did not differ significantly among HIV-positive men who ever had an AIDS-defining disease in the past (CDC stage C) vs those who had CDC stage A.
The MCPyV DNA load was determined from all samples positive for MCPyV DNA on sPCR or on nPCR (Table 3). The MCPyV DNA load ranged between 0.00 and 14,650.00 in all swab samples analyzed (median, 0.98; mean, 117.00). In samples showing MCPyV DNA positivity only on nPCR, MCPyV DNA loads ranged between 0.00 and 95.14 (median, 0.25; mean, 3.30); samples showing MCPyV DNA positivity both on sPCR and nPCR had MCPyV DNA loads between 0.01 and 14,650.00 (median, 8.57; mean, 286.97). The MCPyV DNA loads in HIV-positive MSM (median, 0.75; mean, 133.92) were not significantly higher cutaneous MCPyV DNA loads than those with well-controlled HIV infection (median, 0.48; mean, 11.84) (P = .046). As found for MCPyV DNA prevalence, current smoking status had no influence on MCPyV DNA load, either among HIV-negative men or in HIV-positive men. In HIV-negative men, the median MCPyV DNA loads were 1.23 in nonsmokers (n = 68), 1.00 in smokers (n = 27), and 1.37 (n = 23) in those with unknown smoking status (P = .90). In HIV-positive men, the median MCPyV DNA loads were 0.66 in nonsmokers (n = 75) and 1.96 in smokers (n = 49) (P = .98).

Polymaviridae are a family of small nonenveloped double-stranded DNA viruses that can cause disease in immunocompetent individuals (possibly mild respiratory infections by KI polyomavirus and WU polyomavirus) and in immunosuppressed individuals (BK polyomavirus–induced cystitis or nephropathy and JC polyomavirus–induced leukoencephalopathy) (reviewed by Jiang et al33). The MCPyV is the probable cause of most MCC. Integration of the viral genome into the host genome and expression of the viral oncoregiment antigen are hallmarks of MCPyV-induced MCC development.1,17,21,34-37 On the other hand, studies24,27,38-41 on MCPyV DNA and antibody prevalence have shown that MCPyV is widespread among the general population and is acquired early in life. The association of MCPyV with skin tumors other than MCC is not very likely: because frequencies of MCPyV DNA prevalence among these other tumors do not exceed those of normal healthy skin, viral DNA loads are significantly lower than those in MCC, and viral oncoregiments are not expressed.12,16,17,19,23,26 Besides on the skin, MCPyV DNA has been detected in lower frequencies among respiratory secretions (1%-17%), on oral and anogenital mucosa (4%-50%), and in the digestive tract (17%-33%).16,19,24,32,42-45 By analyzing skin swabs of more than 400 HIV-positive and HIV-negative men, we found MCPyV DNA on the forehead among 53.9% of all participants, which confirms the high MCPyV prevalence previously documented among normal skin.16,19,24,27 For example, Foulongne et al24 detected MCPyV DNA among 80% of cutaneous swabs of healthy volunteers, and Schowalter et al37 recovered complete circular MCPyV genomes from skin swabs among 40% of healthy adult volunteers. Furthermore, the latter authors showed that MCPyV DNA is chronically shed from skin in the form of assembled virions. The concept that MCPyV is part of the human skin microbiome is supported by serologic studies27,38-41 of MCPyV that found antibodies against MCPyV capsid proteins in 42% to 88% of adults without MCC and in somewhat lower percentages of children.

To our knowledge, this is the first study that investigates cutaneous MCPyV infection in HIV-positive individuals. Previously, MCPyV DNA was detected in 30% to 50% of normal oral and anogenital mucous membrane swabs from HIV-positive MSM,19 and Sharp et al11 analyzed lymphoid tissue biopsy specimens from patients with AIDS who were negative for MCPyV DNA. In the present study, we compared MCPyV DNA preva-

**Table 3. Merkel Cell Polyomavirus (MCPyV) DNA Load Among HIV-Positive MSM and Among Healthy Male Control Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCPyV DNA Load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCPyV DNA Load</td>
</tr>
<tr>
<td>Positivity, No.</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td>HIV-positive MSM</td>
<td>124</td>
</tr>
<tr>
<td>Well-controlled HIV infection</td>
<td>65</td>
</tr>
<tr>
<td>Poorly controlled HIV infection</td>
<td>58</td>
</tr>
<tr>
<td>Healthy male control subjects</td>
<td>118</td>
</tr>
<tr>
<td>Total</td>
<td>242</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; MSM, men who have sex with men.

a Seven of the MSM who had HIV could not be classified as having controlled or uncontrolled HIV infection because of missing data.

or B disease: MCPyV was positive on nPCR in 63.3% (38 of 60) vs 57.6% (83 of 144) of samples (P = .53), and MCPyV was positive on sPCR in 30.0% (18 of 60) vs 27.1% (39 of 144) of samples (P = .73). An explanation for this is that almost all patients (96.7% [58 of 60]) who ever had an AIDS-defining disease were receiving HAART, and more than half of them (55.0% [33 of 60]) had well-controlled HIV infection at the time of swab collection.

Current smoking status had no influence on MCPyV DNA prevalence as measured by nPCR or sPCR, either among HIV-negative men or in HIV-positive men. In HIV-negative men, 48.2% (68 of 141) of nonsmokers, 51.9% (27 of 52) of smokers, and 50.0% (23 of 46) of those with unknown smoking status showed MCPyV DNA positivity on nPCR (P = .90); the respective values on sPCR were 15.6% (22 of 141), 11.5% (6 of 52), and 21.7% (6 of 29) (P = .73). By analyzing skin swabs of more than 400 HIV-positive and HIV-negative men, we found MCPyV DNA on the forehead among 53.9% of all participants, which confirms the high MCPyV prevalence previously documented among normal skin.16,19,24,27 For example, Foulongne et al24 detected MCPyV DNA among 80% of cutaneous swabs of healthy volunteers, and Schowalter et al37 recovered complete circular MCPyV genomes from skin swabs among 40% of healthy adult volunteers. Furthermore, the latter authors showed that MCPyV DNA is chronically shed from skin in the form of assembled virions. The concept that MCPyV is part of the human skin microbiome is supported by serologic studies27,38-41 of MCPyV that found antibodies against MCPyV capsid proteins in 42% to 88% of adults without MCC and in somewhat lower percentages of children.

To our knowledge, this is the first study that investigates cutaneous MCPyV infection in HIV-positive individuals. Previously, MCPyV DNA was detected in 30% to 50% of normal oral and anogenital mucous membrane swabs from HIV-positive MSM,19 and Sharp et al11 analyzed lymphoid tissue biopsy specimens from patients with AIDS who were negative for MCPyV DNA. In the present study, we compared MCPyV DNA preva-
lence and load among forehead skin swabs of HIV-positive MSM and healthy male control subjects. On nPCR and sPCR, men with HIV were significantly more often positive for MCPyV than HIV-negative men. When looking at sPCR (which has a 100-fold lower analytical sensitivity than nPCR) only, HIV-positive men with poorly controlled HIV infection were more than twice as frequently positive for MCPyV than men without HIV. These results point to increased MCPyV shedding in severely immunocompromised patients. Viral load determinations showed that high MCPyV DNA loads can also be found in healthy HIV-negative men, but HIV-positive patients having poorly controlled HIV infection had significantly higher MCPyV DNA loads than those having well-controlled HIV infection. As already shown for human papillomavirus infection among HIV-positive individuals,47,48 HIV-associated immunosuppression seems to be associated with increased MCPyV prevalence and load. In contrast to human papillomavirus infection,59 current smoking status had no influence on MCPyV prevalence or load among HIV-positive men nor among HIV-negative men investigated in our study. In line with this, smoking has been identified as a risk factor for human papillomavirus–induced anal or cervical cancer but not for MCC.5,58 Considering the widespread distribution of MCPyV, MCC development seems to be a rare event.5,58 Compared with the general population, HIV-positive patients have up to a 13-fold increased risk for MCC development, and case reports10,11,31-34 of MCC in HIV-infected individuals have been published. Pastrana et al46 suggested that the development of MCC is preceded by an unusually robust MCPyV infection as measured by strong antibody responses against the virus. In another study,38 significantly more patients with MCC carried antibodies against MCPyV capsid protein than control subjects. Consistent with this, MCPyV DNA prevalence and load among nonlesional skin swabs of patients with MCC were higher than those of healthy volunteers and of patients with other skin diseases.34 Possibly, the increased risk for MCC observed in HIV-positive patients can be explained by elevated cutaneous MCPyV DNA loads in these severely immunocompromised patients, making integration of the MCPyV genome into the host cell genome more likely.

The results presented herein should be interpreted in consideration of the limitations in the study. Only a single swab from one body site was collected from each participant. Therefore, we cannot discern transient and persistent MCPyV infection or transmission from other body sites. We cannot rule out that MCPyV RNA prevalence or load among swabs collected from other skin sites or at different points in time would have been different. Foullonge et al24 collected cutaneous swabs from several skin sites of healthy volunteers and found that MCPyV DNA was more frequently detected among swabs taken from the face than from the trunk or limbs. Therefore, by collecting forehead swabs, we probably did not underestimate cutaneous MCPyV DNA point prevalence. Primarily, men of white race/ethnicity were included in our study. Therefore, the results and conclusions may not be generalizable to other races/ethnicities. A further limitation is that the age distributions among patients with HIV and controls were not identical. Tolstov et al49 showed an age-dependent increase in MCPyV antibody prevalence, while other studies48,50,51 have not found variations by age. We are unaware of studies of age dependence in cutaneous MCPyV DNA prevalence, but the prevalence of cutaneous betapapillomavirus DNA was shown to increase with age.35 Although control subjects were slightly older than HIV-infected men in our study, MCPyV DNA prevalence was still higher among the latter group.

In summary, we showed in a large collective of HIV-positive and HIV-negative men that MCPyV DNA prevalence on forehead skin is increased among HIV-positive men and that HIV-positive men with poorly controlled HIV infection have significantly higher cutaneous MCPyV DNA loads than those with well-controlled HIV infection. Possibly, restoration of immune control by early initiation of HAART could lower the elevated MCC risk observed among patients with AIDS by reducing cutaneous MCPyV replication. On the other hand, prolonged survival times of HIV-infected patients who receive HAART could lead to further increases in MCC incidence, as older age is among the risk factors for MCC development.5,58 In any case, our data support the suggestion by Lanoy et al10 to regularly screen HIV-positive individuals for the presence of MCC.

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REFERENCES

3. Heath M, Jaimes N, Lemos B, et al. Clinical characteristics of Merkel cell carci-