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ORIGINAL INVESTIGATION

High seroprevalence of antibodies against Kaposi's sarcoma-associated herpesvirus (KSHV) among HIV-1-infected children and adolescents in a non-endemic population

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Abstract Human herpesvirus-8 (HHV-8) is the etiological agent of Kaposi's sarcoma (KS), which primarily affects human immunodeficiency virus (HIV)-infected adults with advanced immunodeficiency. Currently, only limited prevalence data for HHV-8 infection in HIV-infected children living in non-endemic areas are available. This multicenter cross-sectional study was conducted in four university hospitals in Germany specializing in pediatric HIV care. Stored serum specimens obtained from 207 vertically HIV-1-infected children and adolescents were tested for antibodies against lytic and latent HHV-8 antigens. Logistic regression was used to assess independent risk factors associated with HHV-8 seropositivity. The overall HHV-8 seroprevalence was 24.6 % ($n = 51/207$) without significant differences related to sex, age, or ethnicity. In univariate analysis, HHV-8 seropositivity was significantly associated with a child having being born outside Germany, maternal origin from sub-Saharan Africa, a history of breastfeeding, CDC immunologic category 3, and deferred initiation of antiretroviral therapy (>24 months of age). In multivariate analysis, a child's birth outside Germany was the only significant risk factor for HHV-8 seropositivity (odds ratio 3.98; 95 % confidence interval 1.27–12.42). HHV-8-associated malignancies were uncommon; only one patient had a history of KS. Serum specimen of vertically HIV-infected children and adolescents living in Germany showed a high HHV-8 seroprevalence. These findings suggest that primary HHV-8 infection—a risk factor for KS and other HHV-8-associated malignancies—occurs early in life. Thus, management of perinatally HIV-infected children should include testing for HHV-8 coinfection and should consider future risks of HHV-8-associated malignancies.

Keywords HIV-1 · Human herpesvirus-8 · HHV-8 · Kaposi's sarcoma-associated herpesvirus · KSHV · Pediatric patients · Kaposi's sarcoma

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Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8), is the etiological agent of all forms of Kaposi's sarcoma (KS), primary effusion lymphoma, and multicentric Castleman's disease [1–3]. HHV-8 is a human gammaherpesvirus, which infects B-lymphocytes, endothelial cells, macrophages, and epithelial cells [4]. Like other herpesviruses, HHV-8 exists in both lytic and latent forms and results in chronic infection [5]. Advanced immunodeficiency in HIV-infected individuals is the main risk factor for the development of HHV-8-associated malignancies. However, a substantial proportion of KS cases now occur in HIV-infected patients with relatively high CD4 cell counts [6, 7].

The detection of antibodies to lytic and/or latent HHV-8 antigens indicates a previous HHV-8 infection. Reactivation or viral replication is characterized by high levels of lytic antibody titers, and enhanced lytic replication with high lytic antibody titers is associated with the development of KS [8]. Prevalence of HHV-8 antibodies shows a wide global variation. HHV-8 infection is highly prevalent in sub-Saharan Africa with rates of more than 50 % among the adult population [9]. Children living in endemic areas usually acquire HHV-8 infection early in life, reaching adult seroprevalence rates before puberty [10–12]. Saliva appears to be a source of infectious virus, and it has been proposed that transmission in prepubertal children occurs mainly through saliva of HHV-8-infected close household contacts [11, 13, 14]. Vertical transmission seems to play a limited role [15, 16].

In the USA and most European countries, HHV-8 infection usually occurs after puberty, and HHV-8 seroprevalence rates are lower than 10 % in the general population [17, 18]. For healthy children, seroprevalence rates of 3–4 % have been reported in the USA and Germany [19].

However, little is known about the extent of HHV-8 infection in HIV-infected children living in non-endemic areas.

The aim of the present study was to determine the seroprevalence of HHV-8 among vertically HIV-1-infected pediatric patients living in Germany and to evaluate its association with HIV disease, age, gender, ethnicity, and other demographic factors.

Methods

Patients

From January 2012 to May 2013, a multicenter cross-sectional study was performed in four specialized care centers for HIV-infected children in Germany (Charité, University Medicine Berlin; Goethe University, Frankfurt/Main; Ludwig-Maximilians-University, Munich; and the Medical Faculty Mannheim, Heidelberg University).

Stored serum samples from 207 HIV-1-infected children (aged >12 months) and adolescents were tested for HHV-8 antibodies. Included were all vertically HIV-infected patients, who were regularly seen at one of the sites and from whom at least one stored serum sample was available. Specimens from patients in Frankfurt ($n = 51$) had been collected between February 2001 and March 2009, whereas serum samples from Mannheim ($n = 36$), Munich ($n = 52$), and Berlin ($n = 68$) were collected between January 2007 and May 2013. These patients were defined as the "study group." Demographic and medical data from all patients were recorded in a pseudonymized form at each site.

To evaluate the potential influence of HIV infection on the acquisition of HHV-8, the seroprevalence in the study group was compared with that in a control group of HIV-uninfected children with a similar range of ethnic and socioeconomic backgrounds. For this purpose, serum was analyzed from 56 consecutive HIV-exposed but uninfected children who were seen between January 2012 and March 2013 at the outpatient clinic for pediatric HIV, Charité University Medicine Berlin. The children were followed from birth until HIV transmission was definitely excluded. Serum samples were only available from the last visit at age 16–22 months.

Written informed consent to diagnostic procedures and use of medical records for research according to guidelines approved by each individual university hospital was obtained from all parents of children aged <18 years and all patients ≥18 years of age. The study was approved by the local institutional ethics committees.

Measurements

Serum specimens were tested by indirect immunofluorescence assay for IgG antibodies to lytic HHV-8 antigens (encoded by ORF65) and latent antigens (LANA-1 encoded by ORF73) using a body cavity B-cell lymphoma-1-based cell line [20] as previously described [21]. All tests were performed at the Institute of Medical Virology, Charité University Medicine, by a single technician blinded to patient details. Specimens with antibody titers of at least 1:16 were classified as HHV-8 antibody-positive, whereas titers below 1:16 were categorized as HHV-8 antibody-negative. Subjects with a positive test result on lytic and/or latent HHV-8 antibody assay were considered to be HHV-8-seropositive.

Study parameters

Additional data collected for this analysis include continent of maternal origin, patient's country of birth, ethnicity,

sex, and age at the time of sampling, as well as virologic, immunologic, and clinical characteristics, current combination antiretroviral therapy (cART), and start of initial antiretroviral therapy (ART) in patients with a treatment history. The use of at least three antiretroviral drugs (ARVs) was classified as cART. CD4 T cell percentage (CD4 %) was chosen as an age-independent marker to describe the patient's current immunologic status and was categorized according to the Center of Disease Control and Prevention (CDC) HIV infection classification system as severe (<15 %), moderate (15–24 %), or no evidence of immunosuppression (≥25 %) [22]. CD4 % and HIV-1 viral load were determined concurrently or within 12 weeks of the date of serum sampling.

Initiation of ART was defined as the time point of starting treatment with at least two ARVs. ART initiation up to the age of 24 months was defined as "early treatment," whereas ART initiation after the age of 24 months was termed "deferred treatment." No data on HHV-8 serostatus were available for mothers or other family members.

Children were classified as HIV-exposed but uninfected if they were born to an HIV-infected woman, and a negative HIV antibody test was documented at 12 months of age or later.

Statistical analyses

Categorical variables were compared using the Fisher's exact and Pearson χ^2 tests, and continuous variables were analyzed by the Mann–Whitney U test. Multivariate logistic regression analysis was performed to calculate odds ratios (OR) and 95 % confidence intervals (CI). Variables found to be significantly associated with HHV-8 infection in univariate analysis were included in the model. All p values are two-tailed. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using Stata version SE 12.1 (StataCorp, College Station, Texas, USA).

Results

A total of 207 perinatally HIV-infected children and adolescents were included in the study group. Their baseline characteristics are summarized in Table 1.

The majority of patients were born in Germany (61.4 %), but nearly half of their mothers originated from sub-Saharan Africa (45.9 %). At the time of serum sampling, 92.3 % of the patients were on cART, only 5.3 % had never been treated for HIV infection. Data on ART initiation were available for 188 of 207 children. Of these patients, 88 of 188 (46.8 %) started ART within the first 24 months of life and 72 infants (38.3 %) within the first

12 months; first ART was initiated at a median age of 28 months (IQR 5–69.5). HIV-1 viral load was <50 copies/ mL in 56.9 % of the patients at the time of serum sampling. HHV-8-associated malignancies were uncommon; only one child from Chechnya had a history of KS at 2 years of age. In this boy, who presented with a massive generalized lymphadenopathy, hepatosplenomegaly, and high-grade fevers, an advanced stage of disease was diagnosed—chemotherapy and effective cART resulted in a sustained remission.

The control group consisted of 56 HIV-1-exposed but uninfected children with a median age of 1.5 years (IQR: 1.50–1.58). All had received perinatal HIV transmission prophylaxis with zidovudine for 28 days, were non-breast-fed, and HIV infection was excluded by a confirmed negative HIV antibody test. Children's baseline characteristics are shown in Table 1.

The patients in the study and control group showed the same distribution of ethnicities. However, all children in the control group were born in Germany and received care in accordance with the standardized procedures defined by the German protocol for the prevention of mother-to-child transmission of HIV [23]. In particular, mothers did not breastfeed, and there was a higher level of Cesarean section delivery than in the study group.

HHV-8 seroprevalence

Overall, 24.6 % (51/207) of the patients in the study group had HHV-8-specific antibodies against latently and/or lytically expressed antigens. There was no difference between male and female participants and no age-dependent trend or a significant difference between the age groups ($p = 0.531$) (Fig. 1). HIV-infected children with African or mixed (African/Caucasian) ethnicity had a higher seroprevalence (33/111; 29.7 %) compared to the other patients, with Caucasian, Asian, or Hispanic ethnicity (18/96; 18.8 %), but this did not reach statistical significance ($p = 0.076$). A highly significant risk factor for HHV-8 seropositivity in univariate analysis was birth outside Germany ($p < 0.0001$), due to a high seropositivity of 47.1 % (24/51) in children born in sub-Saharan Africa compared to 15.0 % (19/127) in children born in Germany ($p < 0.0001$). Higher HHV-8 seropositivity rates were also associated with a maternal origin in sub-Saharan Africa ($p = 0.012$), a history of breastfeeding ($p = 0.027$), and a CDC immunologic category 3 ($p = 0.028$) (Table 2). Children who had received initial ART at the age of two years or later had a higher HHV-8 seroprevalence compared to those who were treated earlier ($p = 0.003$).

Children with CD4 % of <25 % tended to show a higher seroprevalence rate (14/36; 38.9 %) than those who had a CD4 % of ≥25 % (37/168; 22.0 %) (Fig. 2). In addition, we noted that children on current cART were less often

Table 1 Baseline characteristics of HIV-infected children and adolescents (study group) and control group of HIV-exposed, uninfected children

	Study group (n = 207)	Control group (n = 56)	p value
Sex [no. (%)]			
Female	107 (51.7)	30 (54.5)	0.762
Male	100 (48.3)	25 (45.5)	
Age at serum sampling (years)			
Median	10.5	1.5	<0.0001
IQR	6–15	[1.5–1.6]	
Born in Germany [no. (%)]	127 (61.4)	56 (100)	<0.0001
Born outside Germany [no. (%)]	80 (38.6)	0 (0)	<0.0001
Sub-Saharan Africa	51 (24.6)	–	
Europe other than Germany	11 (5.3)	–	
Other countries	18 (8.7)	–	
Ethnicity [no. (%)]			
Caucasian	78 (37.7)	20 (35.7)	0.648
African	83 (40.1)	27 (48.2)	
Mixed (African/Caucasian)	28 (13.5)	7 (12.5)	
Asian, Hispanic	18 (8.7)	2 (3.6)	
Maternal origin [no. (%)]			
Sub-Saharan Africa	95 (45.9)	31 (55.4)	0.025
Western Europe	71 (34.3)	11 (19.6)	
Eastern Europe	19 (9.2)	11 (19.6)	
Other geographical origin	22 (10.6)	3 (5.4)	
Mode of delivery [no. (%)]			
Vaginal	131/186 (70.4)	9 (16.1)	<0.0001
Cesarean section	55/186 (29.6)	47 (83.9)	
Breastfed [no. (%)]	99/161 (61.5)	0 (0)	<0.0001
Treatment history [no. (%)]			
Currently receiving cART	191 (92.3)	N/A	
Ever received ART	195 (94.2)	N/A	
CDC immunologic category 3[a] [no. (%)]	74/202 (36.6)	N/A	
History of AIDS (CDC clinical category C)[a] [no. (%)]	39/202 (19.3)	N/A	
Age at first ART (months)			
Median	28	N/A	
IQR	5–69.5	N/A	
CD4+ count (cells/mm^3)[b]			
Median	810	–	
IQR	552–1112	–	
CD4+ T cell percentage[b]			
Median	35	–	
IQR	28–42	–	
HIV-1 viral load < 50 copies/mL[b] [no. (%)]	116/204 (56.9)	N/A	

IQR interquartile range, *cART* combination antiretroviral therapy, *N/A* not applicable

[a] CDC (Center for Disease Control and Prevention) clinical stage of pediatric HIV infection: C, severely symptomatic; CDC immunologic category 3, severe immunosuppression

[b] Laboratory values at the time of HHV-8 serology testing

seropositive for HHV-8 than untreated subjects ($p = 0.075$). The rate of HHV-8 infection in children with deferred initiation of ART was 2.3 times higher when compared to those with early treatment (OR 2.31; 95 % CI 0.86–6.16). However, this finding did not reach statistical significance ($p = 0.095$). In the multivariate logistic regression analysis,
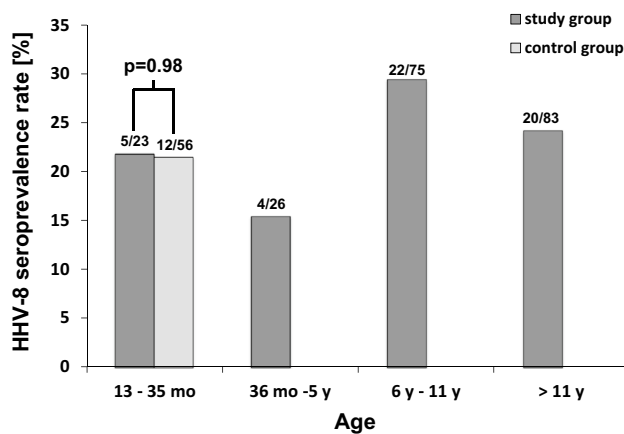
Fig. 1 HHV-8 seroprevalence by age in the study group ($n = 207$) and control group ($n = 56$). The age is given in months (mo) or years (yr), the number of positive/number of tested patients is shown above each *bar* (*p* value: χ^2 test)

the child's country of birth other than Germany was the only significant risk factor (OR 3.53; 95 % CI 1.22–10.26) for HHV-8 seropositivity in HIV-infected children and adolescents (Table 3).

In order to clarify the influence of concomitant HIV infection on HHV-8 seroprevalence, we compared data of the study group with a control group of HIV-exposed uninfected children. In our study, there was no significant difference in the prevalence of HHV-8 antibodies (specific antibodies against latently and/or lytically expressed antigens) between the HIV-infected study group (51/207; 24.6 %) and the children in the control group (12/56; 21.4 %). This also applies if only HIV-infected patients aged 13–35 months are considered (5/23; 21.7 %).

HHV-8 antibody titer among HIV-infected children and control group

The distribution of HHV-8 lytic and latent antibody titers of seropositive subjects stratified by HIV-1 infection status is shown in Fig. 3a, b. Overall, lytic HHV-8 antibody titers were higher in the HIV-positive population than in the control group ($p = 0.005$). The distribution of latent HHV-8 antibody titers, on the other hand, did not differ significantly ($p = 0.49$). Interestingly, the proportion of HHV-8-infected children with both lytic and latent antibodies was significantly higher in the HIV-positive group (82.4 vs. 41.7 %; $p = 0.008$).

Discussion

We found a high HHV-8 seroprevalence among children born to HIV-infected mothers: Nearly one-quarter were

HHV-8-seropositive irrespective of their own HIV infection status.

This seroprevalence is significantly higher than rates reported for the general pediatric population living in non-endemic areas. Martro et al. [19] found a low rate of three to four percent in 787 children and adolescents living in the USA and Germany. Similarly, a low rate of 1.1 % was observed in a large cross-sectional study from the USA of more than 4000 healthy children, aged 6–17 years [24].

Only a few studies have investigated HHV-8 seroprevalence in HIV-infected children in settings in which HHV-8 infection is non-endemic, and to our knowledge none since the introduction of cART. A study from the USA with a small cohort of 51 HIV-1-infected children with AIDS conducted in the pre-cART era found no evidence of HHV-8 infection [25].

In our study, perinatally HIV-infected patients who were born in a country other than Germany, mainly in regions in which HHV-8 infection is endemic, had a significantly higher HHV-8 seroprevalence compared to those born in Germany. Children who were born to mothers from sub-Saharan Africa and who had a history of breastfeeding were more likely to be HHV-8-seropositive. Although we did not have data on HHV-8 infection status of mothers or other family contacts, it is highly probable that mothers originating from endemic regions were more likely to be HHV-8-infected and are the main source of infection. Studies from the UK and Africa show that vertical transmission appears to play only a limited role, even in children of HIV-coinfected mothers [15, 16, 26] with HHV-8-exposed infants losing maternal antibodies within the first year of life [15]. Transmission through breast milk seems to be unlikely, as shown in studies from Zambia [27, 28]. But HHV-8 is frequently found in the saliva of infected individuals, irrespective of their HIV infection status [27, 29]. We therefore postulate that transmission in our cohort occurred horizontally through close contact with HHV-8-infected individuals. Our findings suggest that the main risk factor for HHV-8 infection in children born to HIV-infected mothers is HHV-8 exposure due to close family contacts.

Also at increased risk were HIV-infected individuals with a history of and/or current immunodeficiency. Furthermore, HIV-infected children who had received early ART which maintained their immunological function were less likely to be HHV-8-seropositive. These findings are consistent with studies demonstrating the impact of immunodeficiency on the HHV-8 seroprevalence in HIV-infected adults [8, 30], and they highlight the importance of maintaining an efficient immune status in order to reduce the risk of HHV-8 infection and subsequent development of KS and other HHV-8-associated malignancies.

In univariate analysis, we found a number of risk factors for HHV-8 infection in HIV-infected children and

Table 2 Risk factors for seropositivity to HHV-8 (univariate analysis)

	HHV-8 seroprevalence Study group (n = 207)	p value	HHV-8 seroprevalence Control group (n = 56)	p value
Total [no. (%)]	51/207 (24.6)		12/56 (21.4)	
Sex [no. (%)]				
Female	29/107 (27.1)	0.423	9/30 (30)	0.189
Male	22/100 (22.0)		3/25 (12)	
Ethnicity [no. (%)]				
African/Mixed	33/111 (29.7)	0.076	7/33 (22.2)	0.962
Caucasian/Asian/Hispanic	18/96 (18.8)		5/23 (21.8)	
Country of birth [no. (%)]				
Germany	19/127 (15.0)	<0.0001	12/56 (21.4)	N/A
Other countries	32/80 (40.0)		0	
Maternal origin [no. (%)]				
Western Europe	11/71 (15.5)	0.012	4/11 (36.4)	0.41
Sub-Saharan Africa	31/95 (32.6)		6/31 (19.4)	
Mode of delivery [no. (%)]				
C-section	9/55 (16.4)	0.095	9/47 (19.2)	0.39
Vaginal	38/131 (29.0)		3/9 (33.3)	
History of breastfeeding [no. (%)]				
No	10/62 (16.1)	0.027	12/56 (21.4)	N/A
Yes	32/99 (32.3)		0	
CDC category [no. (%)]				
Clinical category C	11/39 (28.2)	0.544	–	
Clinical category N/A/B	40/168 (23.8)		–	
Immunologic category 3	25/74 (33.8)	0.028	–	
Immunologic category 1/2	25/128 (19.5)		–	
Receiving current cART				
Yes	44/191 (23.0)	0.075	–	
No	7/16 (42.1)		–	
Age at first ART [no. (%)]				
≤24 months	12/88 (13.6)	0.003	–	
>24 months	32/100 (32.0)		–	
CD4 %[a] [no. (%)]				
<25 %	14/36 (38.9)	0.054	–	
≥25 %	37/168 (22.0)		–	
HIV-1 viral load[a] [no. (%)]				
<50 copies/mL	28/116 (24.1)	0.723	–	
>50 copies/mL	23/88 (26.1)		–	

CDC (Center for Disease Control and Prevention) clinical stage of pediatric HIV infection: N, no symptoms; A, mildly symptomatic; B, moderately symptomatic; C, severely symptomatic; CDC immunologic category 1, no immunosuppression; 2, moderate immunosuppression; 3, severe immunosuppression

cART Combination antiretroviral therapy, *ART* antiretroviral therapy, *N/A* not applicable

[a] Laboratory values at the time of HHV-8 serology testing

adolescents; however, a multivariate analysis showed that birth outside Germany was the only independent risk factor for HHV-8 infection in our cohort.

We found high HHV-8 seroprevalence rates in all age groups, suggesting a primary HHV-8 infection in early childhood. Even in young children aged 13–35 months born to HIV-infected mothers, we found HHV-8 antibodies in approximately 20 % of the patients (Fig. 1). The persistence of high antibody titers in older children may be explained by ongoing viral acquisition and/or viral reactivation.
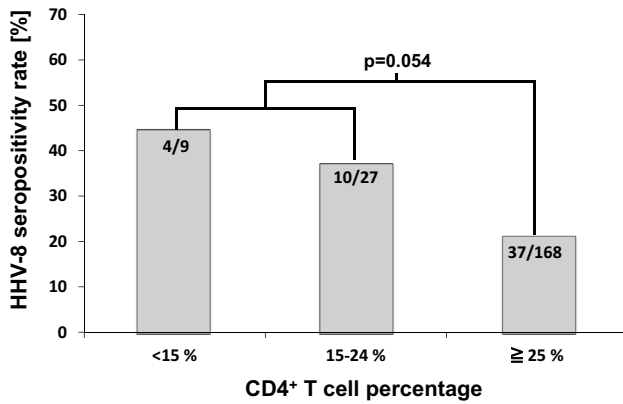
Fig. 2 Proportion (%) of HHV-8-seropositive patients ($n = 51$) of the study group stratified by current CD4 percentage. The number of positive/number tested are shown inside each *bar*

Our data did not show an association between HHV-8 infection and the HIV infection status. This finding is in contrast to other studies in pediatric populations, mainly conducted in sub-Saharan Africa [31–33]. An explanation for this may lie in the large proportion of our patient population on cART. Furthermore, although children in the study and control groups were all born to HIV-infected women and had a comparable range of ethnic backgrounds, they were not matched by age.

A well-controlled HHV-8 infection is characterized by high latent and low lytic antibody titers, whereas the opposite is seen during reactivation or viral replication. The risk of KS seems to be increased with enhanced lytic replication, as reflected by high lytic antibody titers [8]. Although HHV-8 seropositivity rates were similar between the study and control group, we found significantly higher antibody titers against lytic antigen in the HIV-infected study group. This finding is consistent with other studies performed in pediatric and adult populations [30, 31] and may be related to immunosuppression. In HHV-8-infected children with sufficiently controlled HHV-8 replication, a decrease in HHV-8 antibodies is expected resulting in low

or undetectable antibody concentrations. Loss of immune control due to HIV infection may lead to an HHV-8 viral load increase with a subsequent immune response to HHV-8 replication resulting in higher HHV-8 antibody titers.

Few studies in adults have demonstrated an inverse correlation between the severity of the immunodeficiency and level of HHV-8 lytic antibody titers [8, 30].

Despite almost 25 % of our study group being HHV-8-coinfected, HHV-8-associated malignancies were uncommon, with only one child with KS at 2 years of age.

Although HHV-8 infection precedes the development of KS, it is not fully understood how it leads to neoplastic disease, and it has been proposed that additional cofactors might be relevant [34]. Considering that HIV-associated KS in children frequently presents as a generalized lymphadenopathy [35, 36], which is also a common symptom of pediatric HIV infection, and since more than 90 % of patients in our study group received effective cART with potential subsequent regression of KS without specific therapy, a significant proportion of KS might have gone undiagnosed. Studies have shown clinical improvement of KS in HIV-infected adults with immune reconstitution following cART without chemotherapy [37, 38]. Patients of our study group received the first ART early in life, which might lower the risk of KS manifestation in HHV-8-positive subjects.

In the USA and Europe, KS is rare even in HIV-infected children, particularly since the introduction of cART [39]. However, with vertically HIV-infected children now surviving to adulthood, concerns about the future risk of KS or other malignancies later in life have become more relevant. A recently published study from Denmark describes a two-fold increased risk of cancers related to smoking and viral infections, but not for other malignancies in HIV-infected patients [40]. Data of a long-term cohort study in the USA observed an increased risk of KS in HIV-infected adults, even in the context of successful cART [6].

To the best of our knowledge, this is the first study determining the seroprevalence of HHV-8 among vertically

Table 3 Logistic regression analyses of risk factors for seropositivity to HHV-8 (study group, $n = 152$)

	OR[a]	95 % CI[b]	*p* value
Birth outside Germany	3.53	1.22–10.26	0.020
Mother from Western Europe versus sub-Saharan Africa	0.77	0.28–2.13	0.618
History of breastfeeding	0.89	0.52–1.51	0.665
ART started at >24 versus ≤24 months of age	2.31	0.86–6.16	0.095
CDC category 3 versus 1/2	1.04	0.76–1.42	0.823

Adjusted for patient's country of birth, maternal geographic origin, history of breastfeeding, age at first ART, and CDC immunologic category 3
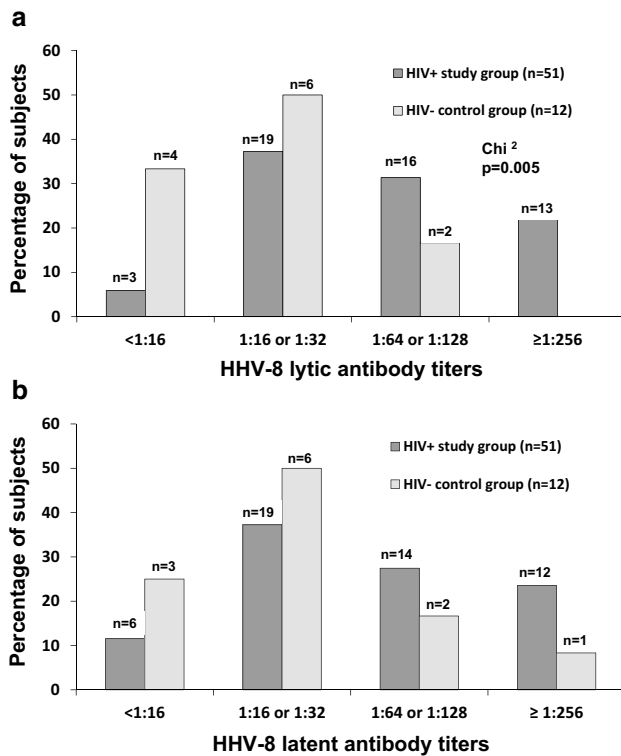
[a] Odds ratio

[b] Confidence interval

Fig. 3 Lytic antibody titers (**a**) and latent antibody titers (**b**) in HHV-8-seropositive (positive for HHV-8 lytic and/or latent antibodies) patients ($n = 63$) stratified by HIV infection status. Titers <1:16 were considered negative. Significant differences in lytic titer levels were found between study patients and control group ($p = 0.005$). The n values above *bars* refer to the overall number of HHV-8-seropositive patients in each titer group

HIV-infected children and adolescents living in a low incidence country in the era of cART. However, there are some limitations to our study. As mentioned above, although the control group offered a good match for background and ethnicity, the age match was not ideal. Furthermore, the cross-sectional study design we used does not provide information on the clinical course of the HHV-8 infection. Finally, we could not identify the route of HHV-8 transmission, since we had no data on maternal HHV-8 serostatus, the family's sociodemographic situation, or the country of paternal origin.

In conclusion, we found a high rate of HHV-8 infection in HIV-infected pediatric patients living in a non-endemic country in Europe. This means that a high proportion of these patients had a significant risk of exposure to HHV-8 and acquired HHV-8 infection early in life. A compromised immune system and a deferred ART initiation are risk factors for HHV-8 infection and enhanced viral replication. These observations emphasize the importance of early initiation of cART in all HIV-infected children to preserve or restore immune function, and ultimately lower the susceptibility for HHV-8 infection and disease progression.

Current guidelines do not recommend routine testing for HHV-8 antibodies [41]. However, the determination of the patient's HHV-8 serostatus should be considered in HIV-infected children presenting with unexplained clinical symptoms consistent with HHV-8 disease.

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