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Correlates of Human Herpesvirus-8 DNA detection among adults in Italy without Kaposi sarcoma

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Background The presence of Human Herpesvirus-8 (HHV8) DNA is predictive of Kaposi sarcoma (KS) among patients with HIV-associated or iatrogenic immunosuppression. However, correlates of HHV8-DNA detection in the general population remain undefined.

Methods We assessed correlates of HHV8-DNA detection among Italian adults without KS who had antibodies against HHV8-latent nuclear antigen by immunofluorescence assay. HHV8-K6 DNA sequences were detected in peripheral blood mononuclear cells using TaqMan PCR.

Results Of the 158 subjects 26 (16.5%) had detectable HHV8-DNA [median copies/million cells, 53; (13–2128)]. Adjusted for age, sex, and laboratory, HHV8-DNA was detected more frequently in participants with >7 total residents in the childhood home [OR = 3.7 (1.5–9.1)], >2 younger siblings [OR = 2.6 (1.1–6.5)], and current cardiovascular [OR = 3.6 (1.3–9.7)] or renal [OR = 3.1 (1.2–8.0)] disease. Excluding the participants using immune modulating drugs, HHV8-DNA was more frequent among those with low red blood cells (RBC) [<4.5 $10^6/\mu l$; OR = 5.3 (1.7–16.2)], slightly elevated mean corpuscular volume [>92 μm^3/red cell; OR = 2.8 (1.0–7.8)], and mild thrombocytopenia [<151 K/μl; OR = 5.6 (1.9–16.3)].

Conclusions Presence of HHV8-DNA in elderly Italians is associated with childhood crowding, low RBCs, and platelets, perhaps indicating roles for early infection and chronic inflammation. These risk factors are the first to be reported for non-immunosuppressed HHV8-seropositive adults.

Keywords HHV8, Kaposi sarcoma herpesvirus, KSHV, viral DNA, viral load

Since its discovery,[1] Human Herpesvirus-8 (HHV8) has been consistently detected in all clinical variants of Kaposi sarcoma (KS)[2,3] including classic (CKS),[4] endemic,[5] iatrogenic,[6] and AIDS-associated forms.[2,3,7] HHV8, also known as Kaposi sarcoma-associated Herpesvirus (KSHV), is a necessary cause of KS but additional cofactors are required for the pathogenesis of this vascular malignancy of lymphatic endothelial origin.[1]

HHV8 seroprevalence is increased among populations at risk for HIV-1 infection[8] and among solid organ transplant recipients.[9–11] In the general population, the seroprevalence of HHV8 demonstrates regional variability irrespective of the type of assay used.[12] Among blood donors from Northern Europe, the detection of HHV8 antibodies against latent nuclear antigen (LANA) and open reading frame 65 is infrequent, ranging between 3 and 5%.[13,14] In contrast, seroprevalence to these antibodies is generally higher in Southern Europe, ranging from 2 to 35%,[15–21] with the highest frequency reported in southern

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Italy.[16] In Italy, the distribution of HHV8 geometric mean titres recapitulates the north–south gradient, with persons seropositive for HHV8-LANA exhibiting higher viral titres in the south (geometric mean HHV8 antibody titre, 11 631) compared with their northern counterparts (geometric mean HHV8 antibody titre, 455).[16]

The transmission of HHV8 infection is not well defined. Serologic studies suggest that it occurs sexually[8,21–27] and by alternative horizontal routes, possibly through saliva[28,29] and blood products.[24] HHV8 transmission in endemic areas begins in childhood[15,19,30–33] and the seroprevalence increases with age to ~50 years,[34] suggesting that transmission can occur throughout life.

Following seroconversion, infection with HHV8 is life-long and may alternate between a lytic and a latent lifecycle.[35] It is not known whether the presence of viral DNA sequences in peripheral blood mononuclear cells (PBMCs) is a result of the reactivation and replication of HHV8 or an expansion of latently infected B cells. Nonetheless, among patients with HIV-associated or iatrogenic immunosuppression, the presence of PBMC HHV8-DNA is predictive of KS.[36] However, correlates of PBMC viral DNA detection in HHV8-infected individuals, who may be at heightened risk for this malignancy, remain undefined. Therefore, we examined correlates of DNA sequence detection among healthy HHV8-seropositive controls in a case–control study of CKS in Italy, an area endemic for HHV8.

Methods

Study population

Controls from the Italian Kaposi sarcoma case–control study (KCC) manifesting anti-HHV8 LANA were used to assess correlates for the detection of HHV8-DNA in PBMCs.[37] As previously published,[37] we enrolled 192 healthy subjects without KS but with evidence of HHV8 latent antibodies. Questionnaire information and PBMC DNA adequate for the HHV8 viral load amplification were available from 158 (82.3%) of these subjects.

All the subjects were healthy volunteers ⩾18 years of age without evidence for KS or HIV-1 infection. They were identified through sampling the patient populations of collaborating local primary care physicians from Sicily as well as mainland sites in Rome and Naples between April 1998 and October 2001. Subjects were screened for HHV8-LANA antibodies.[37] Those testing seropositive by immunofluorescence (IFA) were invited to provide informed consent and enrolled in the KCC.

Data collection

At enrollment, trained personnel obtained the questionnaire data and blood samples from all subjects. Information from questionnaires included demographic, occupational, sexual, medical and family histories, personal hygiene, and alcohol and cigarette use.

Laboratory methods

As previously described,[37] antibodies against HHV8-LANA were detected in a 1:120 dilution of sera by IFA, using the BCBL-1 cell line without tetradecanoyl phorbol-ester acetate induction.[34] Specimens with punctate nuclear immunofluorescence were considered seropositive.

HHV8-DNA load was determined in PBMCs using a quantitative real-time polymerase chain reaction assay. PBMCs were frozen and stored in liquid nitrogen until tested. DNA was extracted from PBMCs using the QIAmp DNA blood kit (Qiagen Inc. Valencia, CA) and the number of copies of HHV8-DNA determined according to the standard procedure.[38–41] Quantitative real-time amplifications were performed in triplicate against the HHV8 K6 gene using the primers 5′-CGCCTAATAGCTGCTGCTACGG-3′ and 5′-TGCATCAGCTG CCTAACCCAG-3′ and the probe 5′-CACCCACCGCCCGTC-CAAATTC-3′ on an ABI Prism 7700 (P.E. Biosystems, Foster City, CA). The number of HHV8 copies detected in triplicate samples were averaged and normalized to the number of PBMCs (copies/10^6 cells), as determined by parallel quantification of the human *ERV-3* gene, using the primers 5′-CATGGGAAGCAAGG-GAACTAATG-3′ and 5′-CCCAGCGAGCAATACAGAATTT-3′, and the labelled probe 5′-(FAM) TCTTCCCTCGAACCTGCAC-CATCAAGTCA(TAMARA)-3′.[42] The lower limit of detection for HHV8 was three copies per million PBMC.

Complete blood counts were performed using the standard protocol. Proportions of peripheral blood T-lymphocyte subsets (helper/inducer and suppressor/cytotoxic) were estimated using flow cytometry on fresh whole blood and monoclonal antibodies for CD4 and CD8, respectively. Absolute numbers of T-lymphocyte subpopulations were estimated as the product of total lymphocytes, from the complete blood count, and the respective T-lymphocyte proportions. All blood studies were analysed at the time of study enrollment in centralized laboratories in Sicily (Dipartimento di Igiene e Microbiologia 'Giuseppe D'Alessandro', Universitá degli studi di Palermo) and Central Italy (Laboratorio di Epidemiologia e Biostatistica, Istituto Superiore di Sanitá, Rome).

Ethical considerations

Institutional Review Boards from each study site reviewed and approved the protocol, the questionnaire, and related materials prior to initiation.

Statistical analysis

Using a cross-sectional approach, we investigated correlates of HHV8-DNA present in PBMCs either due to primary infection or viral reactivation. Based on the distribution among subjects without detectable HHV8-DNA, age was divided into tertiles (⩽70, 71–77, and >77 years of age). Potential differences by geography were examined by collapsing the study site into north (Central Italy) and south (Sicily) to correspond with centralized processing laboratories. Initially, median values of blood cell parameters among subjects without evidence of viral DNA were used to define high and low categories. Based on statistically significant associations with detectable PBMC HHV8-DNA, red blood cells (RBCs) and platelets were examined further by using cutoffs above and below the 15th percentile, a threshold that allowed adequately sized comparison groups while approximating the clinical significance. Detectable HHV8-DNA was dichotomized into two categories, present or absent, where values <10 copies per 10^6 cells were considered absent and values ⩾10 copies per 10^6 cells were considered present. To minimize misclassification, the one study

subject who had eight viral DNA copies per 10^6 cells detected was excluded from our analyses. To evaluate correlates of the HHV8-DNA load, three ordered categories of HHV8-DNA levels were defined as undetectable, low [median 30 copies per million PBMC (range 13–78)], and high [median 157 copies per million PBMC (range 127–2128)], with the high group corresponding to above the 75th percentile. Quartiles were chosen based on graphical examination of the data.

The presence of viral DNA sequences in PBMCs was estimated by the prevalence odds ratio (OR) and the corresponding 95% confidence interval (CI) calculated by the use of logistic regression.[43] Tests for statistical significance of trend were conducted using multiple logistic regression with median levels of the HHV8-DNA load per category as a continuous variable. All estimates were adjusted for potential confounders including age, sex, and the processing laboratory unless otherwise specified. For each variable of interest, final models were selected by comparing the goodness of fit χ^2 for models that included selected additional potential confounders and interaction terms. Statistical significance, based on multivariate logistic models, was calculated with the maximum conditional likelihood test and χ^2. Stratified analyses were used to assess haematologic correlates of HHV8-DNA independent of the processing laboratory. All statistical tests were two-sided. All analyses were conducted in STATA version 7.0 (College Station, TX).

Results

Of the 158 HHV8-seropositive adults without CKS included in this investigation, the majority were male (70%) and from Sicily (79%). The median age did not significantly differ by sex [mean = 73 years (range 37–92) for males and mean = 74 years (range 44–90) for females]. Overall, 26 (16.5%, 95% CI 10.6–22.3%) participants had HHV8-DNA detected in PBMCs. The median level of HHV8-DNA detected in PBMCs was 53 copies/10^6 cells (range 13–2128 copies/10^6 cells). Detection of HHV8-DNA was not statistically significantly different by sex, age (mean = 76 years, HHV8-DNA+ and 72 years, HHV8-DNA−), or the processing laboratory (Table 1). Consistent with the complete study population, the majority of patients with evidence of PBMC HHV8-DNA were male (65.4%) and from Sicily (73.1%).

Table 1 Detection of PBMC HHV8-DNA by sex, age, and laboratory among Italian adults without Kaposi sarcoma

	HHV8 DNA+ (*n* = 26)	**HHV8 DNA− (*n* = 132)**	***P***
Gender			
Male (% HHV8 DNA+)	17 (15.5)	93	
Female (% HHV8 DNA+)	9 (18.8)	39	0.60
Age			
Mean age at interview, years (range)	76 (59–92)	72 (44–89)	0.11
Geography			
Sicily (% HHV8 DNA+)	19 (15.2)	106	
Central Italy (% HHV8 DNA+)	7 (21.2)	26	0.41

The prevalence of HHV8-DNA was higher among persons with current lower socioeconomic status. Crowding in the childhood home, particularly those whose childhood home had more than seven total residents (OR = 3.7, 95% CI 1.5–9.1), more than two younger siblings (OR = 2.6, 95% CI 1.1–6.5), and increasing number of total siblings (*P*-trend = 0.04, Table 2) were associated with detection of PBMC HHV8-DNA. Compared with fewer younger siblings, having more than two younger siblings was associated not only with the detection of PBMC HHV8-DNA but also with high levels of HHV8-DNA (>126 copies/10^6 cells; OR = 4.5, 95% CI 1.1–18.1; *P*-trend = 0.008). Likewise, participants with more than seven residents in their childhood homes were more likely to have high levels of viral DNA (OR = 6.4, 95% CI 1.6–25.2) compared with those with fewer residents (*P*-trend = 0.04).

As shown in Table 2, analyses of current personal hygiene habits, cigarette smoking, and alcohol consumption did not reveal statistically significant associations with HHV8-DNA detection. Among men, the total number of lifetime sex partners did not support a strong association with the detection of PBMC HHV8-DNA. Four women reported more than one lifetime sex partner, of whom three had detectable viral DNA (OR = 29.5, 95% CI 2.2–388). No participant had a history of thalassemia or other haemoglobinopathy. Among participants with cardiovascular or renal disease (Table 3), the probability of detecting HHV8-DNA was statistically elevated (OR = 3.6, 95% CI 1.3–9.7 and OR = 3.1, 95% CI 1.2–8.0, respectively). Prior or current users of corticosteroids were not statistically significantly more likely to have detectable PBMC HHV8-DNA than non-users (OR = 1.1, 95% CI 0.5–2.6), including separate evaluations for each route of administration ($P \geq 0.17$).

We excluded 4 DNA-positive and 17 DNA-negative subjects who used corticosteroids or cancer chemotherapeutics at the time of enrolment for analyses used to examine blood count and T-cell correlates of PBMC HHV8-DNA detection (Table 4). While detection of HHV8-DNA was unrelated to haematocrit and haemoglobin levels ($P \geq 0.26$), it was more common in subjects with RBC and platelet counts below the median (OR = 3.1, 95% CI 1.0–9.4; OR = 4.1, 95% CI 1.4–12.4, respectively) and a mean corpuscular volume (MCV) above the median (OR = 2.8, 95% CI 1.0–7.8). Only four HHV8-DNA positives had clearly low RBC ($<4.2 \times 10^6/\mu l$) or platelet (<100 K/μl) counts precluding our ability to make meaningful conclusions from this analysis. However, among patients with low RBC or platelet counts (redefined at the 15th percentile: RBC $< 4.5 \times 10^6/\mu l$ and platelets < 151 K/μl), the likelihood of detecting HHV8-DNA was markedly elevated (OR = 5.3, 95% CI 1.7–16.2 and OR = 5.6, 95% CI 1.9–16.3, respectively). Compared with controls, increasing levels of viral load were strongly associated with having low RBC (*P*-trend = 0.01) and platelet counts (*P*-trend = 0.02).

In contrast, no notable difference in levels of white blood cell parameters, including total leukocytes, total lymphocytes, and corresponding T-cell subpopulations (CD4+ and CD8+ cells), were seen between participants with and without detectable PBMC HHV8-DNA ($P \geq 0.91$). Similar distributions in the leukocyte differential (neutrophils, basophils, monocytes, and eosinophils) were seen in the two groups ($P \geq 0.30$). Associations were similar using T-cell subpopulations expressed as percentages of the total lymphocyte population (data not shown).

Table 2 Socioeconomic correlates of detectable PBMC HHV8-DNA among Italian adults without Kaposi sarcoma

	HHV8 DNA+		**HHV8 DNA−**			
Variable	*n*	%	*n*	%	**Adjusted OR**[a]	**95% CI**
Education (>5 years)	13	50.0	78	59.1	0.7	0.3–1.6
Rural place of birth[b]	10	38.5	42	31.8	1.5	0.4–5.2
Childhood home						
No indoor toilet	18	69.2	71	53.8	1.9	0.8–4.8
No indoor faucet	15	57.7	51	38.6	2.0	0.8–4.9
>7 total residents	17	65.4	48	36.4	3.7	1.5–9.1
>4 younger residents	19	73.1	77	58.3	3.1	1.1–9.1
>2 older residents	15	57.7	54	40.9	1.9	0.8–4.5
>3 in bedroom	12	46.2	49	37.1	1.5	0.6–3.5
No. of siblings[c]						
0–2	4	15.4	29	22.0	1.0	
3 or 4	4	15.4	40	30.3	0.7	0.2–3.2
5 or 6	8	30.8	32	24.2	2.2	0.6–8.4
>6	10	38.5	31	23.5	2.8	0.8–10.4
No. older and younger siblings[d]						
>2 older siblings	12	46.2	40	30.3	2.1	0.9–5.1
>2 younger siblings	16	61.5	58	43.9	2.6	1.1–6.5
Skin hygiene						
Often very dirty (>10 h/week)	11	42.3	78	59.1	0.5	0.2–1.3
More dirty than neighbours	11	42.3	55	41.7	1.0	0.4–2.4
Bathe or shower <2 times per week	16	61.5	74	56.1	1.1	0.4–2.6
Cigarette smoking[e]						
None	9	34.6	51	38.6	1.0	
Ever	15	57.7	79	59.8	1.3	0.3–6.4
<1 pack/day	7	26.9	45	34.1	1.1	0.2–8.5
≥1 pack/day	8	30.8	34	25.8	1.6	0.3–8.7
<50 pack-years	6	23.1	37	28.0	1.1	0.2–6.3
≥50 pack-years	9	34.6	42	31.8	1.5	0.3–7.7
Alcohol consumption						
Ever	21	80.8	102	77.3	1.3	0.4–4.0
Wine	21	80.8	98	74.2	1.6	0.5–4.8
Liquor or beer	9	34.6	40	30.3	1.3	0.5–3.5
No. lifetime sexual partners						
Men						
Low (1 partner)	1	5.9	6	6.7	1.0	
High (>1 partners)	16	94.1	84	93.3	1.1	0.1–10.0
Women						
Low (1 partner)	6	66.7	38	97.4	1.0	
High (>1 partner)	3	33.3	1	2.6	29.5	2.2–388

Categories defined by the median among the HHV8-DNA negative group. A total of 26 HHV8-DNA positives and 132 HHV8-DNA negatives were included. Column percentages may not add up to 100 due to missing data.

[a] Adjusted for tertile of age (≤70, 71–77, and >77 years), sex, and processing laboratory.

[b] Place of birth refers to urban or rural province in Italy (urban is defined as census areas containing > 100 000 persons).

[c] Increasing number of total siblings (older and younger) was significantly associated with the detection of PBMC HHV8-DNA ($P = 0.04$).

[d] Inceasing numbers of younger siblings by category (0–1, 2–3, and >3) was significantly associated with the detection of PBMC HHV8 DNA ($P = 0.008$), whereas increasing numbers of older siblings by category (0–1, 2–3, and >3) was not signficantly related to the presence of active virus ($P = 0.79$).

[e] Restricted to males.

In multivariate analysis, interactions of age and platelets with the processing laboratory were observed (Table 5). After restricting analyses to participants in Sicily and adjusting for age and sex, a history of having greater than seven total residents in the childhood home (OR = 4.0, 95% CI 1.2–13.8) and platelet and RBC counts below the medians (OR = 5.5, 95% CI 1.3–23.3 and OR = 8.6, 95% CI 1.7–42.8, respectively) confirmed significant univariate associations with the detection of PBMC HHV8-DNA. Sparse data from central Italy (*n* = 4 HHV8-DNA positive cases) prohibited interpretation of multivariate analyses among this group.

Table 3 Comorbidities and steroid use among Italian adults with and without detectable PBMC HHV8-DNA

Variable	HHV8 DNA+ (*n* = 26)	HHV8 DNA− (*n* = 132)	Adjusted OR[a]	95% CI*
Comorbidities				
Allergy	2	21	0.4	0.1–2.0
Arthritis	3	16	0.8	0.2–3.2
Asthma	1	8	0.7	0.1–5.7
Bronchitis or emphysema	9	30	2.0	0.8–5.2
Cancer, non-KS	1	14	0.3	0.1–2.8
Cardiovascular	17	56	3.6	1.3–9.7
Crohns or ulcerative colitis	1	9	0.6	0.1–5.0
Diabetes mellitus	3	23	0.6	0.2–2.2
Gastrointestinal, general	5	21	4.8	0.5–51.0
Gout	0	4	0.0	ND
Liver cirrhosis	1	14	0.4	0.04–3.1
Musculoskeletal	14	61	1.6	0.6–4.0
Other endocrine	0	1	0.0	ND
Other respiratory	1	3	1.9	0.2–20.1
Prostate	1	6	0.3	0.02–4.9
Renal	9	20	3.1	1.2–8.0
Sexually transmitted diseases	3	15	1.2	0.3–4.8
Thyroid	0	5	0.0	ND
Steroid use				
Ever use	10	48	1.1	0.5–2.6
Oral	2	16	0.6	0.1–2.9
Topical	0	18	0.0	ND
Injection	5	15	2.3	0.7–7.3
Inhalation	6	18	2.0	0.7–5.7

ND, not defined.

[a] Adjusted for tertile of age (£70, 71–77, and > 77 years), sex, and processing laboratory.

[b] Columns add up to greater than the total number of study subjects due to multiple diagnoses or modes of steroid use.

Table 4 Haematologic correlates of detectable PBMC HHV8-DNA among Italian adults without Kaposi sarcoma

Variable	HHV8 DNA + (*n* = 22)	HHV8 DNA− (*n* = 115)	Adjusted OR[a]	95% CI
Red blood cells (10^6/μl)				
High (⩾4.95)	5	55	1.0	
Low (<4.95)	17	57	3.1	1.0–9.4
Hematocrit (%)				
High (⩾44.6)	7	55	1.0	
Low (<44.6)	15	57	1.9	0.7–5.6
Hemoglobin (g/dl)				
High (⩾14.6)	8	59	1.0	
Low (<14.6)	14	52	1.9	0.7–5.5
Mean corpuscular volume (μm^3/red cell)				
Low (<92)	16	56	1.0	
High (⩾92)	6	56	2.8	1.0–7.9
Mean corpuscular hemoglobin concentration (g·Hb/dl)				
Low (<32.7)	7	57	1.0	
High (⩾32.7)	15	54	2.3	0.9–6.1
Mean corpuscular hemoglobin (pg/cell)				
High (⩾30)	13	58	1.0	
Low (<30)	9	53	0.7	0.3–1.8
Platelets (K/μl)				
High (⩾214)	5	56	1.0	
Low (<214)	17	52	4.1	1.4–12.4
Leukocytes (K/μl)				
High (⩾6660)	11	56	1.0	
Low (<6660)	11	56	1.0	0.4–2.5
Total Lymphocytes (cells/μl)				
High (⩾1818)	11	56	1.0	
Low (<1818)	11	55	1.0	0.4–2.5
Neutrophils (cells/μl)				
High (⩾3876)	13	56	1.0	
Low (<3876)	9	55	0.7	0.3–1.7
Eosinophils (cells/μl)				
High (⩾126)	10	55	1.0	
Low (<126)	11	50	1.2	0.5–3.1
Basophils (cells/μl)				
High (⩾42)	12	53	1.0	
Low (<42)	9	52	0.8	0.3–2.0
Monocytes (cells/μl)				
High (⩾417)	13	53	1.0	
Low (<417)	8	53	0.6	0.2–1.6
CD4 lymphocytes (cells/μl)				
High (⩾774)	9	55	1.0	
Low (< 774)	10	55	1.0	0.4–2.9
CD8 lymphocytes (cells/μl)				
High (⩾418)	9	55	1.0	
Low (<418)	10	55	1.0	0.4–2.9
CD4:CD8 ratio				
High (⩾1.92)	11	55	1.0	
Low (<1.92)	8	55	0.7	0.3–2.1

Categories defined by the median among the PBMC HHV8-DNA-negative group. Numbers do not add up to totals due to missing data.

[a] Adjusted for tertile of age (⩽70, 71–77, and >77 years), sex, and processing laboratory.

Table 5 Multivariate models of covariates and detection of PBMC HHV8-DNA

Covariate	Model 1 (Full)	Model 2 (Rome & Naples Laboratory)	Model 3 (Sicily Laboratory)
>7 total residents	4.2 (1.4–12.4)	5.6 (0.2–196)	4.0 (1.2–13.8)
Red blood cells (<4.95 10^6/μl)	3.6 (1.1–11.9)	0.6 (0.02–16.6)	5.5 (1.3–23.3)
Platelets (<214 K/μl)	3.9 (1.2–12.0)	0.5 (0.03–8.4)	8.6 (1.7–42.8)
Tertile of age	1.2 (0.6–2.2)	7.9 (0.8–78.0)	0.7 (0.3–1.6)
Male sex	1.0 (0.3–3.0)	1.3 (0.04–45.9)	0.8 (0.2–3.1)
Processing laboratory	1.3 (0.3–4.8)		

Four cases and 22 controls were analysed from Rome and Naples, whereas 18 cases and 93 controls were analysed from Sicily.

Discussion

We conducted a cross-sectional study among HHV8-LANA seropositive persons to measure correlates of detectable PBMC HHV8-DNA, a known risk factor for KS. Evidence from this study of HHV8-LANA seropositive and HIV-1 seronegative persons suggests that the detection of HHV8-DNA is associated with childhood crowding, low RBC and platelet counts, and a normal or slightly elevated MCV. We found significant independent associations of viral DNA and cardiovascular and renal disease, but the presence of DNA sequences in PBMC was not statistically significantly related to correlates of sexual transmission or with markers of T-cell suppression. To our knowledge, this is the first report on correlates of HHV8-DNA detection among healthy persons with latent HHV8-infection in the absence of KS from an area endemic for HHV8 infection.

The frequency of HHV8-DNA detection in PBMCs (16.5%) is consistent with previous findings of HHV8-DNA in HIV-1 negative Italians without KS (0–23%).[44–46] However, the interpretation of published reports is limited because it is not clear whether HHV8-negative individuals, as determined by antibodies against both lytic and latent antigens, were included in the calculation of HHV8-DNA prevalence, which could attenuate estimates and potentially account for the wide range of reported values.

Positive associations of HHV8-DNA associated with large families and, in particular, a close contact with younger children in the childhood home suggest a role for timing of initial infection and risk of high HHV8 viral load as an adult. HHV8 transmission in Italy is not uncommon in childhood,[32] and HHV8 seroprevalence increases linearly with age until ~50 years.[34] Studies in other endemic areas,[31] including Israel[30] and Africa,[47,48] suggest a strong correlation between the HHV8 serostatus of young children and that of their mother, and to a lesser extent, the serostatus of other infected relatives living in the household.[48] These reports suggest that close contact in the childhood home and salivary exposure are important for the transmission of HHV8 infection.[30–33,49,50]

Following primary HHV8 infection, levels of both lytic and latent antibodies typically rise together in a linear fashion.[51] However, it is not clear whether HHV8-DNA levels change over time relative to the alternating lytic and latent HHV8 lifecycle or in the presence of genetic and environmental factors related to immune competence. The quantification of PBMC HHV8-DNA load among HIV-seropositives is generally 10- to 1000-fold higher than patients without HIV infection.[46,52] However, in HIV-seronegative subjects, we did not observe any association between the presence of HHV8-DNA sequences in PBMCs and absolute numbers or percentages of lymphocytes or T-lymphocyte subpopulations, including CD4+ and CD8+ cells.

However, we did detect HHV8-DNA more often in the PBMCs of persons with evidence of low RBCs and normal MCV, perhaps indicative of a mild normocytic anemia ($P = 0.02$). Anaemia can be indicative of a chronic vascular disease or inflammatory process[53] and is, at least in part, due to excessive production of pro-inflammatory cytokines that inhibit the effect of erythropoietin.[54] Laboratory evidence from BC-3 and BCBL-1 primary effusion lymphoma B-cell lines presented by Davis *et al.*,[55] suggest that anemia-induced chronic hypoxia increases the expression of lytic HHV8 proteins, consistent with the reactivation of virus. Thus, it is possible that individuals with anaemia are more likely to actively shed HHV8. Consistent with this observation, we detected PBMC HHV8-DNA ~three times more often in individuals who typically manifest anaemia, including subjects with cardiovascular (OR = 3.6) or renal disease (OR = 3.1), compared with those in whom these comorbidities were absent.

In addition, we detected HHV8-DNA more often in the PBMCs of persons with low total platelets suggesting that platelets may be involved in the reactivation of HHV8 infection or in a cross-reactivity of antibodies formed in response to HHV8 infection. In a parallel investigation, risk for CKS was increased in persons with a low platelet count after controlling for HHV8-DNA, age, sex, and the processing laboratory (E. E. Brown, unpublished data). Together these findings suggest that platelets may contribute to the disease process either directly in the causal pathway or indirectly as a result of a more generalized inflammatory response possibly precipitated by HHV8 reactivation.[56]

In summary, childhood crowding, low RBCs, and mild thrombocytopenia were associated with detection of PBMC HHV8-DNA in elderly Italians, indicating a role of early infection and perhaps reflecting the importance of inflammation. While these correlates may be associated with the presence of PBMC HHV8-DNA, either due to primary infection or reactivation, our investigation is limited to cross-sectional interpretation and confirmation is required in a larger, similarly endemic population. These risk factors are the first to be reported for the detection of PBMC HHV8-DNA in healthy HHV8-seropositive adults without KS or HIV-infection.

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