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Measurements of human herpesvirus 8 viral load in blood before and after leukoreduction filtration

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Abstract

BACKGROUND: Human herpesvirus 8 (HHV-8) is likely transmitted through blood transfusion in high-prevalence areas. The efficacy of leukoreduction filtration for reducing HHV-8 in blood has not been reported.

STUDY DESIGN AND METHODS: Blood was drawn from 45 human immunodeficiency virus–positive men either with Kaposi's sarcoma (KS; n = 21) or without KS (n = 24) and subject to leukoreduction filtration. HHV-8 viral load was measured in plasma and in blood before and after filtration.

RESULTS: Twelve subjects, all with KS, had detectable HHV-8 viremia before filtration with viral loads of 10^2 to 10^5 copies/mL (mean, 3×10^4 copies/mL). After filtration, seven of 12 subjects no longer had detectable HHV-8 in their blood, and five of 12 subjects had detectable HHV-8 that was 90% reduced on average from prefiltration levels. The presence of HHV-8 in the blood after filtration was strongly associated with prefiltration viral loads greater than 1000 copies/mL and the presence of cell-free virus in plasma. None of the subjects without KS had detectable levels of HHV-8 virus in blood before or after filtration.

CONCLUSION: Cell-associated HHV-8 appeared to be effectively removed by leukoreduction filtration. Cell-free HHV-8 was present in 42% of subjects as 1% to 20% of the total virus which was not removed by filtration.

Human herpesvirus 8 (HHV-8) causes Kaposi's sarcoma (KS), a malignancy that occurs primarily in conjunction with AIDS, and is endemic in regions of sub-Saharan Africa.[1] In a study in Uganda, HHV-8 infection appeared to result from blood transfusion in 2.8% of 595 HHV-8–seropositive units transfused.[2] Blood transfusions in Uganda would present a higher risk for transmission of HHV-8 than in most other settings due to high HHV-8 seroprevalence among blood donors (40%), no leukoreduction, and short storage times for blood. The reason for relatively low transmission from HHV-8–seropositive donors is not understood but likely includes the rarity of detectable HHV-8 virus in

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

immunocompetent individuals and the low-titer, periodic HHV-8 viremia often observed in human immunodeficiency virus (HIV)-infected individuals.[3]

In the United States, HHV-8 seroprevalence among blood donors is low at 3% to 5%.[4] Studies in US cohorts before routine leukoreduction indicate HHV-8 transmission by blood transfusion may have occurred but was rare.[5] Since blood-borne HHV-8 is thought to be primarily cell associated, it was presumed that leukoreduction filtration should substantially reduce any risk of infection by blood transfusion. This study explores this hypothesis by measuring HHV-8 viral load in blood collected from subjects coinfected with HIV, before and after leukoreduction filtration, to observe whether filtration efficiently reduces blood-borne HHV-8.

MATERIALS AND METHODS

Specimen collection

Prior approval for this study was obtained from the Centers for Disease Control and Prevention (CDC) and Emory University Institutional Review Boards. Enrollment took place at the Emory HIV/AIDS Clinical Trials Unit in Atlanta, Georgia. HIV-positive men with and without KS who were being seen at the clinic for their medical care were presented with information about the study and the opportunity to enroll which entailed a single blood draw of 50 mL. The enrollment goal was 50 patients with the expectation that at least 20 would be HHV-8 positive and approximately half of those (10) would have detectable HHV-8 viremia. Blood samples were collected in ACD vacutainers suitable for hematologic, serologic, and molecular testing (Becton Dickinson, Franklin Lakes, NJ). Blood samples were transported usually the same day they were drawn and no later than the following day to the Emory blood processing laboratory where all samples were leukoreduced the same day as they arrived. White blood cell (WBC) counts were performed by the Emory clinical hematology laboratory.

Filtration of blood

From 50 mL of whole, peripheral blood collected from each patient, two aliquots were removed before filtration: 0.5 mL for HHV-8 viral load measurement and 1.0 mL for centrifugation to produce plasma for HHV-8 viral load and antibody measurements.

Filtration

Remaining peripheral blood samples were leukoreduced using a sterile pediatric leukoreduction filter (Purecell Neo, PALL Biomedical Products, East Hills, NY). A third aliquot of blood was removed for HHV-8 viral load measurement after filtration. Leukoreduction efficacy was confirmed by flow cytometric enumeration of residual WBCs (Leukocount, BD Biosciences, San Jose, CA) and by performing polymerase chain reaction (PCR) for the cellular gene RNase P on blood samples before and after filtration. Samples were stored at −80°C the same day as leukoreduction and kept there until transport to the CDC laboratory weekly.

HHV-8 PCR and antibody testing

Total DNA was purified from unfiltered blood, filtered blood, and plasma specimens using blood mini kits (Qiagen, Valencia, CA) with typical yields of DNA of 80% to 90% according to the manufacturer and consistent with in-house validations (data not shown). TaqMan-based PCR was performed for HHV-8 DNA targeting the viral ORF 25 region with an analytical sensitivity of five copies of viral DNA per PCR procedure.[6] Immunoglobulin G antibody to HHV-8 was measured in plasma by whole cell immunofluorescence assay.[5]

Statistical analysis

The association between HHV-8 viral load and CD4 was examined using two-tailed Spearman rank correlation test.

RESULTS

Forty-five HIV-positive patients were enrolled, 21 with KS and 24 without KS. All 21 patients with KS were HHV-8 antibody positive; eight of the 24 (33%) patients without KS were HHV-8 antibody positive. Before leukoreduction filtration, 12 of the 21 (57%) KS patients had detectable levels of HHV-8 in their blood with viral loads ranging from 1.8×10^2 to 2.4×10^5 copies HHV-8 DNA/mL (median, 1.1×10^3; mean, 2.7×10^4). After filtration, five of the 21 (24%) KS patients had detectable HHV-8 in their blood at levels that were on average 1 log lower than prefiltration levels and similar to levels measured in their plasma (Fig. 1). None of the 24 patients without KS had detectable HHV-8 DNA in blood before or after filtration or in plasma.

Cell-associated versus cell-free HHV-8 DNA

Six patients had detectable HHV-8 DNA in their plasma, five of whom had remaining HHV-8 viremia after leukore-duction filtration at levels similar to those seen in their plasma samples (Fig. 1). The portion of HHV-8 virus that was cell free (virus copies/mL in plasma divided by virus copies/mL in unfiltered whole blood) ranged from 1% to 20% with a mean of 8%.

Cellular reduction by leukoreduction filtration

The number of WBCs was measured before and after filtration by quantitative PCR on the cellular gene RNase P and showed an average 3-log reduction in the number of WBCs per sample (range, 2.6×10^2-4.3×10^3; median, 2.0×10^3; mean, 1.8×10^3).

For the 12 patients who had detectable HHV-8 in blood, there was a significant inverse relationship between CD4 count and HHV-8 viral load (Spearman's rank coefficient, −0.615; $p = 0.03$) (Table 1). Mean CD4 counts were lower in subjects with KS compared to subjects without KS (226 versus 257; $p = 0.04$).

DISCUSSION

This is the first study to our knowledge to measure HHV-8 viral load in blood before and after leukoreduction filtration. Among 45 HIV-positive subjects, 29 subjects were

positive for HHV-8 antibody and 12 of them, all with KS, had measurable HHV-8 viremia. Leukoreduction filtration reduced WBC levels in blood on average by 3 logs (99.9% reduction) as expected. Filtration removed HHV-8 virus down to undetectable levels in seven of 12 (58%) subjects, all of whom had prefiltration HHV-8 viral loads below 1000 copies/mL and no detectable virus in plasma. Five of 12 (42%) subjects with viral loads greater than 1000 copies/mL and measurable virus in plasma before filtration had detectable HHV-8 in blood after filtration at levels reduced by a mean of 90%.

Our observation of cell-free HHV-8 in subjects with KS and higher viral loads may not be generalizable to blood donors. In this study and other studies higher HHV-8 viral loads and cell-free virus are associated with active KS and disease progression[7-9] and have not been observed in immunocompetent individuals. Two studies performed PCR testing on a total of 58 HHV-8 seropositive US blood donor units[4,10] and no HHV-8 DNA was detected. A third study induced peripheral blood mononuclear cells and CD19+ B cells from 164 blood donors and no HHV-8 was detected indicating the level of HHV-8 in blood donors is extremely low, if present at all.[11] Limitations of our study include small sample size and the inability to measure efficacy of leukoreduction filtration on blood from healthier study subjects without KS since none of them had detectable HHV-8 DNA.

In summary, leukoreduction filtration reduced HHV-8 viral loads to either undetectable levels or by an average of 90% in all HHV-8-positive units examined. Filtration would thus likely substantially reduce any risk of HHV-8 transmission from HHV-8–seropositive blood donors who would presumably have low viral loads and mainly cell-associated virus. The possible risk of cell-free virus in healthy individuals should be directly examined.

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ABBREVIATION:

HHV-8	human herpesvirus 8

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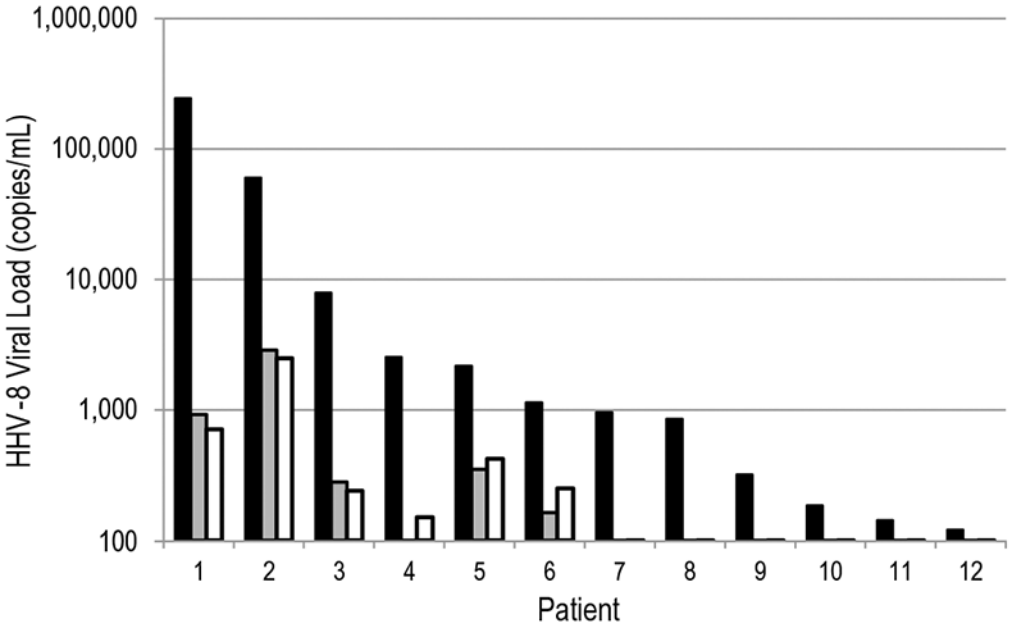

Fig. 1.

HHV-8 viral load in blood before and after WBC filtration and in plasma derived from unfiltered blood. The HHV-8 PCR limit of detection is approximately 100 copies/mL.(■) Unfiltered blood; (▒) filtered blood;(□) plasma.

TABLE 1.

CD4 count and HHV-8 viral load*

		HHV-8 (copies/mL)	
Patient	**CD4 count**	**Before filtration**	**After filtration**
10	767	185	0
8	650	850	0
6	562	1,137	163
12	305	110	0
9	248	319	0
1	152	244,150	920
11	147	142	0
7	132	954	0
5	92	2,168	350
4	67	2,540	0
2	18	60,010	2876
3	14	7,865	279

* KS patients who had detectable HHV-8 in blood listed according to decreasing CD4 cell count, showing an inverse relationship between CD4 cell count and prefiltration viral load ($p = 0.03$).