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Inherited HHV-6A reactivation linked to impaired immune reconstitution following hematopoietic stem cell transplantation

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Whether inherited chromosomally integrated human herpesvirus 6 (eHHV-6 or iciHHV-6) contributes to morbidity[1,2] or represents exclusively a benign genomic trait remains controversial[3,4]. In the setting of transplantation, complications have been mostly documented in graft-mediated horizontal eHHV-6 transmission[5-8]; however, our recent findings indicate that reactivation of recipient-derived eHHV-6 poses a comparable risk to graft outcomes[9].

Here, we describe the clinical course of a hematopoietic stem cell transplant (HSCT) recipient, born with eHHV-6A, in whom reactivation of the virus led to significant comorbidity and impaired immune reconstitution.

At birth, the patient was diagnosed with infant B-cell acute lymphoblastic leukemia with *KMT2A::AFF1* rearrangement, following a presentation of “blueberry muffin” purpuric skin lesions (Figure 1A) and leukocytosis (53 cells x E9/L). The Interfant-21 treatment protocol was started at six days of age; however, induction was complicated by intestinal obstruction, *Candida spp*. sepsis and jugular vein thrombosis. A 28-day course of blinatumomab was subsequently administered, which was complicated by severe cytokine release syndrome that responded to tocilizumab. At two months of age, markedly elevated loads of HHV-6 were detected in blood (6.9×10^6 copies/mL), which were confirmed as maternally inherited.

At five months of age, due to poor treatment response, the patient received an αβ T-cell/CD19-depleted haploidentical graft containing 25 million/Kg of CD34+ cells and 71 000/kg αβ T-cells from the father. This was administered following conditioning with busulfan, fludarabine, thiotepa, anti-thymocyte globulin, and rituximab. The patient had no detectable minimal residual disease (MRD) in bone marrow and no leukemic cells in cerebrospinal fluid (CSF). While neutrophil engraftment occurred rapidly (day +20) and full donor chimerism was achieved, the patient developed persistent thrombocytopenia requiring regular platelet transfusions. One month post-HSCT, the bone marrow was MRD negative with normocellularity and normal megakaryocytes, suggesting increased peripheral platelet consumption rather than impaired production.

Three weeks post-HSCT, defibrotide was initiated for suspected veno-occlusive disease (VOD), which was subsequently excluded via liver ultrasound; similarly, disseminated intravascular coagulation (DIC) and thrombotic microangiopathy (TMA) were ruled out.

Post-HSCT, the clinical course was marked by irritability, weight gain, mild renal dysfunction with macroscopic hematuria, and polyserositis (pericardial and pulmonary edema). No bacterial infections were identified. The patient received acyclovir prophylaxis and was screened weekly for cytomegalovirus (HCMV), adenovirus (AdV) and Epstein-Barr virus (EBV) by PCR, with negative results. The plasma and urine samples were negative for BK polyomavirus by PCR. The HHV-6A DNA peaked in blood at $8.4x10^5$ copies /mL and was concurrently detected in the cerebrospinal fluid (CSF) at $1.7x10^3$ copies/mL. The leukemia was in remission based on the bone marrow and CSF.

On day +92, the patient developed pericardial fluid (PF) build-up with impending tamponade, requiring emergency drainage. The SC5b-9 levels were negative, making thrombotic microangiopathy unlikely, and no leukemic cells were detected in PF. Persistent irritability and pain prompted opioid treatment. Liver values showed mildly elevated alanine aminotransferase (ALT) and marked elevation of gamma-glutamyl transferase (GGT). The patient had sinus tachycardia and severe hyponatremia of uncertain etiology. The lymphocyte counts remained extremely low ($CD3^+$ 0.01, $CD4^+$ 0.003 cells x E9/L) at 3 months post-HSCT. The HHV-6A copy numbers were $6.2X10^5$/mL.

Due to poor graft function and stagnant immune reconstitution, while requiring regular platelet and red blood cell (RBC) transfusions, the patient received a CD34+ booster (10 million cells/kg) at day +110, followed by ganciclovir at day +119. Post-antiviral therapy, the HHV-6A loads dropped dramatically, coinciding with rapid clinical resolution of the edema, hyponatremia, and irritability. The graft function improved markedly, with decreased transfusion requirements and a striking increase in lymphocyte counts. The patient did not receive systemic corticosteroids or other immunomodulatory therapies that could have influenced the clinical course. During the 4 weeks of

GCV treatment, the HHV-6A copy numbers decreased from 4.7x 10^5/mL to 18X10^3/mL. The patient required no further transfusions after discharge five months post-HSCT.

At 2.2 years post-HSCT, the patient remained in full remission, with normalized hematological and biochemical parameters. The HHV-6A copy numbers were 920/mL with relatively good immune reconstitution ($CD3^+$ 0.95 E9; $CD4^+$ 0.557 E9; CD8+ 0.32 E9; and CD19+ 0.88 E9 cells/L).

The dramatic resolution of multiple systemic clinical manifestations, including irritability, and the improved immune reconstitution following GCV and the stem cell boost, suggested a pathogenic for eHHV-6A in this patient.

We therefore retrospectively investigated the role of eHHV-6A in pre- and post-HSCT samples from the patient and parents, using quantitative PCR[10] and targeted viral sequencing of 41 DNA viruses[11]. Informed consent was obtained and the study reviewed by the ethics committee of the Helsinki and Uusimaa Hospital District (statement HUS_462_2021).

Pre- and post-HSCT, blood eHHV6-A copy numbers fluctuated between 6.9 ×10^6/mL and 4 ×10^5/mL, with a sharp decline after the cell booster and GCV administration, inversely correlating with rising lymphocyte counts (Figure 1B).

As the residual clinical samples were inadequate for RNA or protein analysis[9], we performed three alternative approaches to assess reactivation, namely demethylation, viral DNA digestion, and minor variant analyses.

The demethylation (DM) analysis relies on the assumption that integrated eHHV-6 genomes are methylated during latency while demethylated during lytic replication (reactivation). The assay was performed using the NEBnext Microbiome DNA Enrichment kit (New England Biolabs) according to protocol, except for the use of 3X excess of MDB2-fc magnetic beads.

In the samples pre-HSCT, we detected high levels of methylated virus in both the patient and the mother (Figure 1C). Post-HSCT, the unmethylated-to-methylated viral ratio shifted from 0.12 to 18,

subsequently decreasing to 3.6 after the CD34+ booster and GCV therapy. High levels of unmethylated DNA in the pericardial fluid (PF) further supported systemic reactivation (Figure 1C).

To confirm the presence of viral particles in PF, we digested the free DNA using TURBO DNase according to protocol (Thermo Fisher). In PF, we detected $9X10^5$ copies/mL after DNase treatment (from an original $7.6X10^6$copies/mL), consistent with the presence of encapsidated viral DNA (Figure 1C).

To further validate the transition from latency to reactivation, we investigated the emergence of minor variants. These low-frequency subpopulations arise from mutational events occurring during the lytic cycle and thus serve as indicators of active replication. To this end, we performed targeted sequencing of 41 DNA viruses on longitudinal blood samples (n=7), CSF, and PF from the patient, in addition to blood samples from each parent, as previously described[11].

We obtained mean breadth coverages of eHHV-6A of 95.7% (range 81,4-99,1%) and depths of 93X (range 9.8- 160.2X) across the patient samples. Among the other DNA viruses targeted, only anelloviruses were detected. The mother was HHV-6A+, while the father was negative for HHV-6A/B and only positive for HHV-7 (~7% breadth).

Pre-HSCT, the patient´s HHV-6A sequences were identical to the maternal germline. Post-HSCT, however, five distinct viral haplotypes emerged, including three non-synonymous changes in the viral capsid triplex protein 2 gene: g.89449C>T;p.159M>I, g.89452C>A;p.158E>D, g.89463C>G/T;p.155E>Q/K (Figure 1D). The minor viral variants were detected using a dual caller approach with LoFreq[12] and FreeBayes. Only concordant variants with ≥3% frequency and ≥6 supporting reads were retained after quality filtering. Additional corrections and stringent region filters were applied to reduce sequencing artifacts. The germline variant, which represented 100% of the population at baseline, declined to 7.3% post-HSCT as the g.89463C>G variant became dominant. A significant contraction in viral diversity followed the dual intervention of GCV and the stem cell booster. This pattern reflects a transition to controlled replication, potentially driven by the synergy between direct antiviral activity and emerging immune-mediated clearance.

While the precise therapeutic weight of each intervention is difficult to determine, we contend that both were clinically necessary. The dramatic resolution of symptoms compatible with HHV-6 disease immediately following GCV administration provided strong clinical evidence of active viral involvement. We hypothesize that suppressing viral replication was essential to mitigate cytopathicity, facilitating a permissive environment for the stem-cell booster to drive immune reconstitution. Ultimately, we provide evidence that eHHV-6 can reactivate, with clinically significant consequences, underscoring the importance of its consideration in post-transplant risk assessment. Consequently, documenting reactivation is essential to guide clinical interventions, such as antiviral therapy, which may be warranted in patients with neurological symptoms, poor graft function, or exceptionally delayed immune reconstitution[13-15].

While the detection of viral transcripts (e.g. mRNA) would have provided further confirmation, our complementary molecular analyses offer reasonable evidence of eHHV-6A reactivation. Furthermore, we demonstrate that viral DNA demethylation is a viable, clinically deployable marker, especially when suboptimal sample quality or nucleic acid degradation preclude reliable detection of viral analytes.

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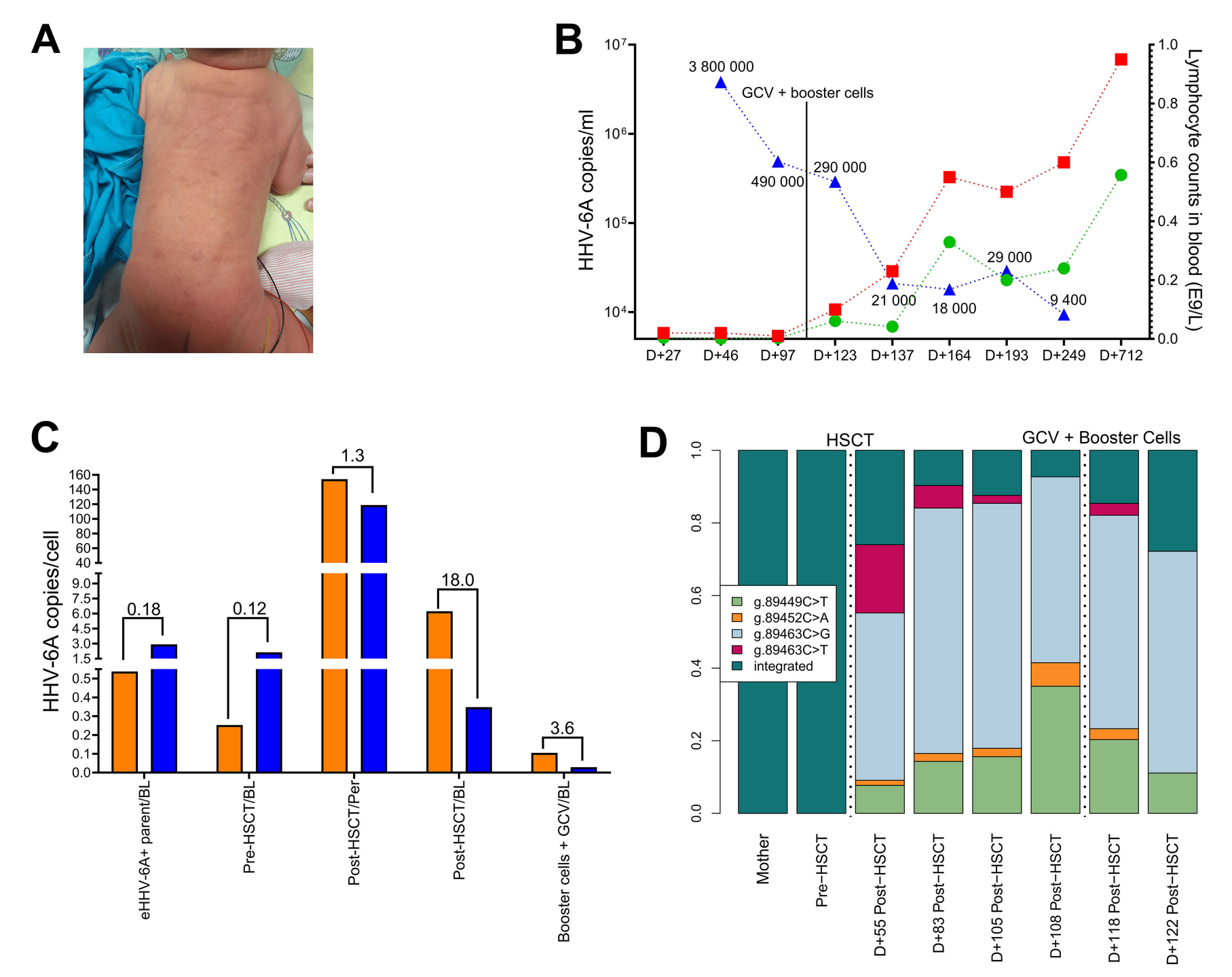
Figure 1. Purpuric lesions discovered during birth and longitudinal eHHV-6A loads and immune reconstitution after HSCT. (A) “Blueberry muffin” rash on the patient´s back. **(B)** Longitudinal (x-axis) monitoring of T-cell subsets and viral load post-HSCT. Absolute T-cell counts for $CD3^+$ (red squares) and $CD4^+$(green circles) cells (10^9/L) are shown on the left y axis; blood eHHV-6A DNA copies/mL in blood (blue triangles) are shown on the right y axis. Vertical line indicates the timing of the stem cell boost and GCV treatment **(C)** Viral DNA methylation status across clinical samples. Bars represent HHV-6A copies/cell (y-axis) in the unmethylated fraction (orange) and unprocessed samples (blue). From left to right, the samples 1–2 correspond to the baseline (pre-HSCT) blood samples of the mother and patient; samples 3–4 to post-HSCT pericardial fluid (Per) and blood (BL), and sample 5 to BL collected post stem cell booster. The calculated unmethylated/methylated ratios are indicated above the corresponding bars; **(D)** Emergence of minor viral variants over time. Frequencies of specific haplotypes (y-axis) within the viral capsid triplex protein 2 are shown, relative to reference NC_001664.4. Nucleotide substitutions and their genomic positions are noted in the left inset. The time points of the transplantations are represented with vertical dotted lines.


A
B
HHV-6A copies/ml
Lymphocyte counts in blood (E9/L)
3 800 000
GCV + booster cells
490 000
290 000
21 000
18 000
29 000
9 400
D+27
D+46
D+97
D+123
D+137
D+164
D+193
D+249
D+712
C
HHV-6A copies/cell
0.18
0.12
1.3
18.0
3.6
eHHV-6A+ parent/BL
Pre-HSCT/BL
Post-HSCT/Per
Post-HSCT/BL
Booster cells + GCV/BL
D
HSCT
GCV + Booster Cells
g.89449C>T
g.89452C>A
g.89463C>G
g.89463C>T
integrated
Mother
Pre−HSCT
D+55 Post−HSCT
D+83 Post−HSCT
D+105 Post−HSCT
D+108 Post−HSCT
D+118 Post−HSCT
D+122 Post−HSCT