An Infant with Severe Combined Immunodeficiency, Osteopetrosis, Chromosomally Integrated Herpesvirus-6 Infection, and Hemophagocytic Syndrome: What are the Links?

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Short running title: Is ciHHV-6 and SCID associated with OP and HPS?

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Abbreviation Key:

Abbreviation	**Full term**
BMA	Bone marrow aspiration
ciHHV6	Chromosomally integrated HHV-6
GvHD	Graft versus Host Disease
HLH	Hemophagocytic lymphohistiocytosis
HPS	Hemophagocytic syndrome
HSCT	Hematopoietic stem cell transplantation
NBS	Newborn screening
OP	Osteopetrosis
SCID	Severe combined immunodeficiency disorder
TA-TMA	Transplant- associated thrombotic microangiopathy
VOD	Veno-occlusive disease

To The Editor:

Osteopetrosis (OP) is a rare and heterogenous group of skeletal dysplasias, characterized by increased bone mineral density secondary to poor bone resorption by dysfunctional/absent osteoclasts with variable clinical spectrum, including pancytopenia [1]. Generally considered to be an inherited disease, a genetic etiology can be established in approximately 90% of cases [2]. Animal experimentation however, has established both genetic and infectious pathways of etiopathology [3].

Human herpesvirus-6 (HHV-6) is a DNA virus with unique ability to integrate into the human genome. Congenital viremia occurs in approximately 1% of the population secondary to chromosomally integrated HHV-6 (ciHHV-6) of parental origin; this form is prone to reactivation during immunodeficiency states [4]. HHV-6 can be associated with hemophagocytic syndrome (HPS) [5]. The implementation of newborn screening (NBS) for severe combined immunodeficiency (SCID) in the USA, has resulted in earlier detection and better prognosis [6]. Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for SCID and restores hematolymphoid system in severe OP. Here we report an infant with SCID, OP, ciHHV-6 infection, and HPS treated successfully with allogeneic HSCT.

A 10-day-old male due with a positive NBS for SCID and persistent thrombocytopenia was transferred for suspected leukemia due to immature cells in the periphery, which were shown to be non-clonal CD34-positive cells that were increased in number with absent T and natural killer (NK) lymphocytes by flow cytometry at our hospital (Figure1A).A pathogenic variant in the interleukin-2 receptor (IL-2R) gamma chain gene confirmed [c.225_226insTGCT (p.Ser26Cysfs*2)] X-linked SCID (T-B+NK-)the diagnosis.

Repeated bone marrow aspirationswere attemptedto evaulate thrombocytopenia,but yielded unsatisfactory samples with very few monocytes and hemophagocytic histiocytes. Subsequently, the patient developed fever and pancytopenia; with elevated ferritin (1251 ng/mL)and LDH (457 IU/mL) levels and was diagnosed with HPS(Figure1B and Figure1C). Serum soluble sIL-2R level(< 31.2) was not elevated.Dexamathasone therapy resulted in resolution of fever. No variants were detected in primary hemophagocytic lymphohistiocytosis genes.

Based on the findings in skeletal survey and high circulating CD45-dim/CD34-positive hematopoietic stem cells (13%), OP was suspected (Figure1A and FigureS1A) [7]. Bone and marrow biopsy sample

obtained by Orthopedics, showed findingsconsistent with OP. MRI of orbits/brain/brainstem was negative [8]. A gene panel evaluating several known OP genes and whole exome sequencing did not reveal any pathogenic variants for OP. Investigating for HPS etiology revealed HHV-6A viremia in blood and cerebrospinal fluid with positive ciHHV-6 test.

Unrelated 10/10-matched donor HSCT was performed following fludarabine 1.2 mg/kg/dose on days -9 to -4, busulfan targeting 65 mg/L x hr equates to an area under the curve (AUC) of 5,278 micromole x min/Lper dose on days -6 to -4 for a cumulative AUC of 15,834micromole x min/Land Thymoglobulin 2.5 mg/kg/dose on days -5 to -3 conditioning with a mononuclear cell dose of $11.35x10^8$/kg and CD34+ cell dose of $7.38x10^6$/kg and graft versus host disease (GvHD) prophylaxis with mycophenolate and tacrolimusat age 5 months. Neutrophil engraftment was established on day +15.Post-transplant course was complicated by worsening HHV-6 viremia, moderate veno-occlusive disease of the liver (VOD), and significant transplant-associated thrombotic microangiopathy (TA-TMA), despite beingon defibrotide prophylaxis for reported high risk VOD in OP [9]. Due to increasing HHV-6 copies, the patient was treated on foscarnet with decreasing viral load. Steroid therapy continued beyond day +100 due to mild skin GvHD.

The patient achieved complete hematopoietic and immune function recovery with significantly lower and stable levels of HHV-6. Repeat radiologic evaluation showed improvement in osseous mineralization along with normal hearing test, ophthalmological examination, and orbital/brain MRI findings (Figure S1B). The patient continues to have mild renal insufficiency as a sequela of TA-TMA, has normal development except speech delay, and shows no evidence of OP one year from HSCT.

The patient's initial presentation, development of subsequent symptoms, investigations leading to series of diagnosis andinterplay between all the conditions resulting in an atypical presentation, are interesting to note in this case. Since the patient had severe lymphopenia secondary to SCID, we

preferred to use the term HPS. Severe T and NK lymphopenia with defective IL-2R may also account for the lack of elevated sIL-2R [10]. In addition to SCID, it is possible that congenital HHV-6 infection could have contributed to HPS development similar to toa single reported case of ci-HHV-6A reactivation, in a patient with X-linked SCID and HPS [11].HSCT was the appropriate therapeutic modality for many conditions the patient had: SCID, osteopetrosis and HPS. Post-HSCT course was interesting with almost simultaneous development of VOD and TA-TMA, even though the patient was on defibrotide prophylaxis. Whether ciHHV-6 infection played a role in this is unclear [12].

We would like to propose a hypothesis linking ciHHV-6 and osteopetrosis, for discussion. Multiple animal models have suggesteda viral etiology for osteopetrosis in differenct species[3, 13-14]. Though retroviruses are RNA viruses, they resemble herpesviruses in their ability to integrate into the human genome and be transmitted via Mendelian inheritance. Osteopetrosis and immunosuppression was reported in newborn kittens infected with feline leukemia virus, that bore a marked resemblance to autosomal dominant OP in humans [3]. Additionally, retroviruses were isolated in adults with benign osteopetrosis [13].Infection with HHV-6 can cause bone marrow suppression in immunocompromised individuals [15]. It is possible that ciHHV-6 with lack of T cell immunity could have led to viremia in utero, which in turn had caused bone marrow failure with decreased/absent osteoclast development resulting in dense bone disease. Decreasing maternal anti-HHV-6 antibodies might have led worsening viremia then to HPS. In conclusion, presence of X-linked SCID and ciHHV-6 infection might have been co-operational in the development of OP and HPS.

Disclosure of Conflicts of Interest

Authors do not have any conflict of interest to report. This manuscript is not under review for publication in another journal.

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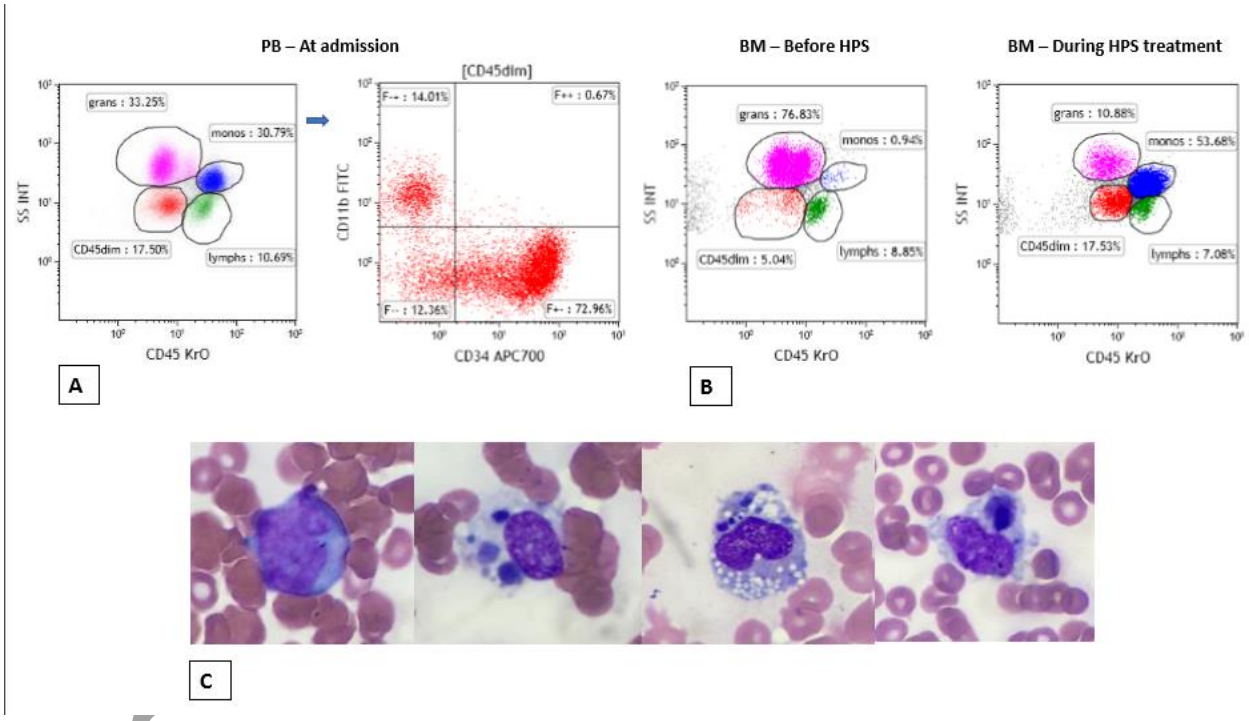

Figure1.

A. Peripheral blood flow cytometric analysis of peripheral blood nuclear cells. Circulating immature cells are located in the CD45-dim population constituting 17.5% of the cells run on the left histogram. CD34-positive and CD11b-negative population makes up 72.9% cells of the gated immature population consistent with hematopoietic stem cells (13%) in the peripheral blood.

B. Bone marrow aspirate flow cytometric analysis show presence of granulocyte population prior to development of hemophagocytic histiocytosis in the significantly acellular sample and increase of monocytic/histiocytic cells during early days of HPS treatment.

C. There was relative prominence of monocytes (1) and hemophagocytic histiocytes (2 – 4) on extremely hypocellular Wright/Giemza-stained bone marrow aspiration smears (100x).