

V348I mutation in UL23 gene of human herpesvirus I in a case of herpetic hepatitis and haemophagocytic lymphohistiocytosis

S. Corcione¹, F. Sidoti², C. Costa², S. Mornese Pinna¹, C. Bonetto³, R. Urbino³, R. Cavallo² and F. G. De Rosa¹

1) Department of Medical Sciences, Infectious Diseases, University of Turin, 2) Department of Public Health and Microbiology, Laboratory of Microbiology and Virology, AOU Città della Salute e della Scienza, University of Turin and 3) Department of Anaesthesia, Intensive Care and Emergency, AOU Città della Salute e della Scienza, Turin, Italy

Abstract

We herein report the case of a young immunocompetent adult patient with a rapidly fatal haemophagocytic lymphohistiocytosis syndrome related to human herpesvirus I (HHV-1) infection, with herpetic hepatitis and persistent high-level viraemia despite treatment with acyclovir. Haemophagocytic lymphohistiocytosis was confirmed in the patient's spleen and bone marrow. HHV-1 DNA was extracted from whole blood and liver biopsy and the UL23 gene was sequenced. A V348I natural polymorphism of the TK protein was found in blood and liver specimens. Further studies are needed to investigate the role of this polymorphism in the development of systemic immune dysregulation. © 2019 The Author(s). Published by Elsevier Ltd.

Keywords: Acyclovir, haemophagocytic lymphohistiocytosis syndrome, UL23 gene, herpetic hepatitis, human herpesvirus-1

Original Submission: 31 December 2018; **Revised Submission:** 27 February 2019; **Accepted:** 5 March 2019

Article published online: 14 March 2019

Corresponding author: S. Corcione, Department of Medical Sciences, Infectious Diseases, University of Turin, Italy.
E-mail: silvia.corcione@unito.it

Introduction

Human herpesvirus (HHV) hepatitis is a rare disease usually affecting immunocompromised patients; it can progress rapidly with severe complications [1]. There are several reports describing the development of haemophagocytic lymphohistiocytosis (HLH) syndrome secondary to primary HHV infections, and these cases are usually associated with a high mortality rate [2]. HLH is a life-threatening condition that results from the uncontrolled activation of the immune system. It was first described in 1939 by Scott et al. [3,4].

Knowledge regarding HLH pathogenesis, clinical presentation, diagnostic criteria and treatment options comes from literature on paediatric patients; thus the prompt recognition of HLH is sometimes difficult [5]. According to the Histiocyte Society, HLH can be classified as primary or secondary [6]:

primary HLH is autosomal recessive and usually presents in childhood, whilst secondary HLH may result from immunological activation by malignancies, autoimmune disorders or infections, of which viruses—particularly human herpesvirus 1 (HHV-1) and human herpesvirus 2 (HHV-2)—are among the most common inducers [7].

Currently, the standard therapy for the management of HHV-1 infections includes acyclovir (ACV). Drug-resistant HHV-1 isolates are frequently recovered from immunocompromised patients. Genotypic characterization of drug-resistant isolates can reveal mutations located in the UL23 gene which encodes the viral thymidine kinase (TK), and mutations in this gene result in resistance to ACV. UL23 gene mutations have been reported in 95% of clinical isolates exhibiting ACV resistance [8]; in particular, these mutations consisted of deletions/insertions or substitutions of nucleotides leading to frameshift reading, resulting in the synthesis of a truncated non-functional enzyme [9]. The most important sites involved in the enzyme activity are the ATP-binding site (aa 51–63), the nucleoside-binding site (aa 168–176), and six highly conserved regions: site 1 (aa 56–62), site 2 (aa 83–88), site 3 (aa 162–164), site 4 (aa 171–173), site 5 (aa 216–222), and site 6 (aa 284–289). In particular, the substrate nucleoside-binding site, the conserved

site 3 and the ATP-binding loop are the sites of the most frequently reported mutations in ACV-resistant clinical isolates [8]. The main mutations associated with ACV-resistance are: R51W, G56S/V, P57H, H58R/L, G59R/V, G61V, K62N, T63A/I/S, D162A, R163H, A168T, P173L/R, A175V and R176Q. Genotypic characterization of HHV-1 clinical isolates revealed a high degree of polymorphism in the UL23 gene outside the active site of the enzyme not associated with resistance [8,10].

We report here the case of a young immunocompetent woman who developed a fatal herpetic hepatitis with persistently high level HHV-1 viraemia, followed by haemophagocytic lymphohistiocytosis syndrome. Moreover, we sequenced the HHV-1 UL23 gene from blood and tissue samples to look for ACV resistance.

Case description

We report here the case of a 22-year-old female admitted to the emergency department of a peripheral hospital with 1-week history of pharyngeal pain, prolonged fever, right-side abdomen tenderness, thick white vaginal discharge, and dysuria partially responsive to antibiotic treatment.

On admission she was febrile (temperature 38.0°C), with pulse 115/minute, respiratory rate 14/minute, and blood pressure 115/70 mmHg. Clinical examination was remarkable for erythematous pharyngitis, bilateral cervical and axillary lymphadenopathy, watery painful blisters and multiple tender vaginal ulcers. On laboratory exams, blood count revealed a reduction in total white blood cells (WBCs) due to absolute neutropenia (neutrophil count 800/mm³) and thrombocytopenia (113 000/mm³); the haemoglobin level was normal.

At the time of admission to the ER, liver function test showed elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to 270 UI/L and 200 UI/L, respectively; cholestasis enzymes and international normalized ratio (INR) were within the reference range. Renal function was within normal limits. Abdominal ultrasound and computed tomography (CT) showed an enlarged liver and spleen with ascites and pelvic effusions consistent with acute hepatitis. Five hours after hospital admission, repeat blood tests showed a

sharp increase in AST and ALT levels to 3000 UI/L and 1300 UI/L, respectively, and with acute hepatitis suspected the patient was transferred to the referral hospital.

At the referral hospital the patient required respiratory support due to progressive dyspnoea, and she was transferred to the intensive care unit (ICU). On clinical exam, she was febrile, dyspnoeic, and laboratory tests revealed a worsening of thrombocytopenia, new onset of renal failure, and further increases in AST and ALT (4200 UI/L and 1300 UI/L, respectively). Chest and abdomen CT scan confirmed mild homogeneous liver and spleen enlargement, mild ascites and initial alveolar pulmonary oedema.

Serological and microbiological tests were performed (Tables 1 and 2), and empirical broad-spectrum antibiotics—piperacillin/tazobactam 4.5 g every 8 h and levofloxacin 750 mg once daily—were started, together with acyclovir 10 mg/kg every 8 h. All exams were negative except for whole-blood real-time PCR targeting HHV-1 DNA polymerase. Blood HHV-1 DNA was >5 000 000 cp/mL, supporting the hypothesis of primary HHV-1 infection with severe related hepatitis. HLH-related HHV-1 infection was suspected as four clinical diagnostic criteria were fulfilled [5]: fever, splenomegaly, leukopenia and thrombocytopenia in the peripheral blood.

Laboratory findings on day 3 from admission indicated sharply worsening clinical features with multiorgan failure, and the patient was started on haemodialysis and supportive treatment. Antibiotic treatment was implemented with vancomycin 1 g every 12 h and caspofungin at standard dosage. A rectal swab was positive for a multidrug-resistant *Acinetobacter baumannii*; blood cultures were repeatedly negative. Blood HHV-1 DNA was persistently high despite intravenous acyclovir (Table 2).

After 10 days of ICU stay, the patient developed an acute respiratory distress syndrome. Bronchoalveolar lavage culture was positive for carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp), and chest radiographs showed extensive right parenchymal consolidation. Due to her progressively worsening condition and multiorgan failure, the patient died on day 15.

TABLE 1. Biochemical analysis performed during the patient's hospital stay

	Day 1 from hospital admission	Day 2	Day 3	Day 5	Day 7	Day 10	Day 15
WBC (10 ⁹ /L)	900	2000	7400	14 000	15 000	18 000	1900
Hb (g/dL)	10.2	10.6	8.6	8.9	8.3	10.4	9.3
PLT (10 ⁹ /L)	91 000	88 000	84 000	51 000	122 000 (T)	71 000	43 000
AST/ALT (UI/L)	807/453	3076/1280	4946/1710	4700/1300	800/274	330/180	200/110
BIL (mg/dL)	1.6	2.2	2.2	3	5	8	11
INR	2.2	1.8	1.7	1.5	1.1	1.1	1.6
PCT (µg/L)	11	12	13	—	6	3	1

WBC, white cell blood count; Hb, haemoglobin; PLTS, platelets; BIL, bilirubin; PCT, procalcitonin; INR, international normalized ratio.

TABLE 2. Microbiological analysis performed during the patient's hospital stay

		Day 1 from hospital admission	Day 5	Day 10	Day 15
HHV-1 DNA (cp/mL)	–	–	5 000 000	5 000 000	–
HHV-2 DNA (cp/mL)	–	–	neg	–	–
HHV-1/2 DNA genital swab	neg	–	–	–	–
<i>Treponema</i> Ig	neg	–	–	–	–
Parvovirus IgM/IgG	neg	–	–	–	–
<i>Brucella</i> IgM/IgG	neg	–	–	–	–
<i>Leishmania</i> Ab	neg	–	–	–	–
CMV DNA (cp/mL)	neg	–	–	–	–
Anti-HCV	neg	–	–	–	–
HBsAg	neg	–	–	–	–
HIV p24/Ab1-2	neg	–	–	–	–
ASLOT (UI/mL)	neg	–	–	–	–
Blood cultures	neg	neg	–	neg	–
Urine culture	neg	neg	neg	Neg	CP-kp CP-Kp; <i>Acinetobacter</i>
Bal	–	–	–	–	MDR Cp-KP
Rectal swab	–	neg	–	–	

ASLOT, antistreptolysin O titre; BAL, bronchoalveolar lavage; CP-Kp, carbapenemase-producing *Klebsiella pneumoniae*; *Acinetobacter* MDR: multi-drug-resistant *Acinetobacter* spp; NEG, negative.

Owing to the rapid progression of the disease, an autopsy was performed. This revealed an enlarged liver consistent with acute liver failure, with multiple foci of confluent necrosis across multiple lobules consistent with HLH; moreover, HLH was confirmed in the spleen and bone-marrow tissue. Immunohistochemical analysis revealed HHV-1 positivity in the liver within a large number of liver-cell nuclei.

Both whole blood and liver biopsy specimens were analysed for HHV-1 mutation conferring resistance to acyclovir.

HHV-1 DNA detection and UL23 gene sequencing

Given the persistently high viraemia, HHV-1 DNA was extracted from whole blood and liver biopsy and the UL23 gene was sequenced.

HHV-1 DNA extraction from whole blood and liver biopsy

Viral DNA was purified from whole-blood specimens using the automated QIASymphony SP/AS instrument (Qiagen, France), according to the manufacturer's instructions. Tissue specimens were formalin-fixed. For DNA extraction, sections (thickness 6 µm) were washed with ethanol and distilled water and incubated with 400 µL of Buffer ATL (Qiagen, France) and 20 µL of

proteinase K (Qiagen, France) at 56°C with shaking at 1000 rpm until the tissue was completely lysed; 200 µL of the supernatant were collected for nucleic acid extraction performed using the QIASymphony SP/AS instrument. In order to quantify the viral load in whole blood, DNA samples were evaluated by 7500 Fast Dx Real Time PCR (Applied Biosystems, Monza, Italy) using the HSV1 ELITe MGB Kit (ELITechGroup, Molecular Diagnostics, Turin, Italy), according to the manufacturer's instructions.

Sequencing of the UL23 gene of HHV-1 from whole-blood and liver-biopsy specimens

Three primer sets, previously optimized in PCR reactions by da Silva and colleagues, were used to amplify the HHV-1 UL23 region (1244 bp) from whole-blood and liver-biopsy specimens [11]. Amplification was performed on a 7500 Fast Dx Real Time PCR instrument (Applied Biosystems, Monza, Italy) under the following conditions: 50 µL reaction volume consisted of 10 µL DNA, 1 x Kapa Taq Ready Mix (0.05 U/µL of Taq DNA polymerase, 2 x reaction buffer with 3.0 mM MgCl₂, 0.4 mM each dNTP; Kapa Biosystems, Boston, MA, USA) and primers 0.3 µM (forward/reverse). The programme for cycling was as follows: denaturation at 95°C for 5 min, followed by 45 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 40 s. The final extension was done at 72°C for 10 min. Amplicons were analysed by electrophoresis on a 2% agarose gel system and PCR products with expected sizes were purified using reagents and protocols of the High Pure PCR Product Purification Kit (Roche, Mannheim, Germany). The resulting purified products were sequenced using the ABI Prism BigDye Terminator version 1.1 Ready Reaction mix (0.5 µL BigDye, 0.75 µL reaction buffer, Applied Biosystems, Foster City, CA, USA). In particular, PCR products were sequenced using the same three primer sets used for amplification (2 µM forward/reverse primers). The programme for cycling was as follows: 25 cycles of 95°C for 10 s, 50°C for 5 s and 60°C for 4 min. Amplicons were capillary sequenced on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies, Carlsbad, CA, USA).

Sequence analysis of the HHV-1 UL23 gene for detection of mutations associated with acyclovir resistance was performed. HHV-1 sequences were manually checked in the BioEdit v. 7.2.5 software. Nucleotide sequence alignments were performed using a reference sequence (strain HSV-1 14-2, GenBank Accession Number: AB078742.1) obtained by NCBI database. Alignments were confirmed using the Mutation Resistance Analyzer (MRA) online HHV-1 drug resistance mutations tool, which compares submitted sequences via BLAST to the reference sequence of UL23. Both whole-blood and liver-biopsy specimens were found to have the same UL23 mutations, in particular the V348I mutation.

Discussion

It is well known that systemic infections can lead to complications such as HLH, and several studies have reported viral infections as the leading infectious triggers of HLH [12–16]. Disseminated HHV-1 infection usually occurs in immunocompromised patients, but has also been described in immunocompetent hosts; reports have described four of five patients with HSV-related HLH as immunocompetent. Clearing the infection trigger is the mainstay to decrease hyperimmune activation in patients with secondary HLH [17]. However, with the exception of HLH associated with human gammaherpesvirus-4, there is little evidence regarding the significance of a combined approach with immunosuppressive therapy in virus-associated HLH, and further data are needed to establish the optimal treatments for virus-associated HLH [18,19].

More recently, a modification in the diagnostic criteria—with three clinical findings plus one immune marker—to achieve diagnosis has been proposed [20]. In our case the presence of fever, pancytopenia, spleen enlargement plus hypertriglyceridaemia fulfilled the diagnosis. Moreover, clinical suspicion was supported by histological confirmation.

Our patient had persistently high viraemia despite the prompt start of acyclovir treatment. Therefore, we sequenced HHV-1 UL23 gene from blood and tissue samples to look for acyclovir resistance, but no mutations known to be associated with acyclovir resistance were reported. Interestingly, we found a V348I mutation that represents a natural polymorphism of the TK protein hitherto not reported as related to acyclovir resistance. Although there are no data available about the clinical relevance of the V348I mutation, our patient had persistently high viraemia and she rapidly developed HLH, suggesting that the V348I mutation could be related to a higher HHV-1 virulence, which may more easily trigger the systemic immune dysregulation.

In conclusion, this is the first report describing a V348I mutation in a patient with disseminated HHV-1 infection, herpetic hepatitis and HLH. So far, there are no data regarding the role of this mutation in clinical practice. More data are therefore needed to correlate this mutation with the rapid and fatal development of HLH related to HHV-1 infection.

Transparency declaration

The authors have no conflicts of interest to declare. No funding was received for the study.

Author contributions

Silvia Corcione and Francesca Sidoti contributed equally to this work.

References

- [1] Farr RW, Short S, Weissman D. Fulminant hepatitis during herpes simplex virus infection in apparently immunocompetent adults: report of two cases and review of the literature. *Clin Infect Dis* 1997;1191–4.
- [2] Brisse E, Wouters CH, Andrei G, Matthys P. How viruses contribute to the pathogenesis of hemophagocytic lymphohistiocytosis. *Front Immunol* 2017;8:1–8. <https://doi.org/10.3389/fimmu.2017.01102>.
- [3] Bodley S, Scott, Robb-Smith R, AHT. Histiocytic medullary reticulosis. *Lancet* 1939;234:194–8.
- [4] Ramos-Casals M, Brito-Zeron P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. *Lancet* 2014;383:1503–16.
- [5] Wysocki CA. Comparing hemophagocytic lymphohistiocytosis in pediatric and adult patients. *Curr Opin Allergy Clin Immunol* 2017;17:405–13. <https://doi.org/10.1097/ACI.0000000000000405>.
- [6] Henter J, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al., for the HS. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis *Pediatr. Blood Cancer* 2007;48:124–31.
- [7] George MR. Hemophagocytic lymphohistiocytosis: review of etiologies and management. *J Blood Med* 2014;69–86.
- [8] Morfin F, Souillet G, Bilger K, Ooka T, Aymard M, Thouvenot D. Genetic characterization of thymidine kinase from acyclovir-resistant and susceptible herpes simplex virus type 1 isolated from bone marrow transplant recipients. *J Infect Dis* 2000;182:290–3.
- [9] Oram RJ, Marcellino D, Strauss D, Gustafson E, Talarico CL, Root AK, et al. Characterization of an acyclovir-resistant herpes simplex virus type 2 strain isolated from a premature neonate. *J Infect Dis* 2000;181:1458–61.
- [10] Kudo E, Shiota H, Naito T, Satake K, Itakura M. Polymorphisms of thymidine kinase gene in herpes simplex virus type 1: analysis of clinical isolates from herpetic keratitis patients and laboratory strains. *J Med Virol* 1998;56:151–8.
- [11] Perse da Silva A, De Oliveira Lopes A, Vieira YR, de Almeida AJ, Sion FS, Grinsztejn B, et al. Genotypic characterization of herpes simplex virus type 1 isolates in immunocompromised patients in Rio de Janeiro, Brazil. *PLoS One* 2015;10(9):e0136825.
- [12] Chevillotte M, Ersing I, Mertens T, von Einem J. Differentiation between polymorphisms and resistance-associated mutations in human cytomegalovirus DNA polymerase. *Antimicrob Ag Chemother* 2010;54:5004–11.
- [13] Otsubo K, Fukumura A, Hirayama M, Morimoto T, Kato M, Mochizuki H. Hemophagocytic lymphohistiocytosis caused by systemic herpes simplex virus type 1 infection: successful treatment with dexamethasone palmitate. *Pediatr Surg Int* 2016;58:390–3.
- [14] Cusini A, Günthard HF, Stussi G, Schwarz U, Fehr T, Grueter E, et al. Hemophagocytic syndrome caused by primary herpes simplex virus 1 infection: report of a first case. *Infection* 2010;38:423–6.
- [15] Honsig C, Beinhardt S, Tomasits J, Dienes HP. Haemophagocytic lymphohistiocytosis associated with fulminant hepatitis and multiorgan failure following primary Epstein–Barr virus and herpes simplex virus type 1 infection. *BMJ Case Rep* 2017. [bcr2016218310](https://doi.org/10.1136/bcr2016218310).

- [16] Ramos-Casals M, Brito-zerón P, López-guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. *Lancet* 2014;383:1503–16.
- [17] Yabushita T, Yoshioka S, Koba Y, Ono Y. Successful treatment of herpes simplex virus (HSV)-I-associated hemophagocytic lymphohistiocytosis (HLH) with acyclovir: a case report and literature review. *Intern Med* 2017;2919–23.
- [18] Marsh RA. Epstein–Barr virus and hemophagocytic lymphohistiocytosis. *Front Immunol* 2017;8:1902.
- [19] Goudarzipour K. Epstein-Barr virus-induced hemophagocytic lymphohistiocytosis. *Int J Hematol Oncol Stem Cell Res* 2013;7:42–5.
- [20] Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. *Blood* 2011;118:4041–52.