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Ultrastructural and Immunohistochemical Diagnosis of a Neonatal Herpes Simplex Virus Infection Presenting as Fulminant Hepatitis: A Case Report

Valentina Papa, Nunzio Cosimo Mario Salfi, Roberta Costa, Ilaria Bettocchi, Emilia Ricci, Duccio Maria Cordelli, Francesca Locatelli, Fabio Caramelli, and Giovanna Cenacchi

Abstract

TORCH (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes Simplex Virus and Syphilis) infections are a major cause of intrauterine and perinatal infections with associated morbidity and mortality. Neonatal Herpes Simplex Virus infection caused by an enveloped, double-stranded DNA virus of the Herpesviridae family is devastating and fatal. Herpes Viruses are not hepatotropic but may rarely cause hepatitis. Most cases of HSV hepatitis rapidly progress to fulminant hepatic failure and often fatal before the diagnosis or transplantation. Nowadays, despite the availability of antiviral

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V. Papa, R. Costa, and G. Cenacchi (✉)
Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy
e-mail: valentina.papa2@unibo.it; r.costa@unibo.it; giovanna.cenacchi@unibo.it

N. C. M. Salfi
Pathology Unit, Integrated Department of Services and Laboratories, Pathological Anatomy Unit, IRCCS Giannina Gaslini Institute, Genova, Italy formerly at Pathology Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy
e-mail: NunzioSalfi@gaslini.org

I. Bettocchi
Pediatric Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy
e-mail: ilaria.bettocchi@aosp.bo.it

E. Ricci
Child Neurology and Psychiatry Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy
e-mail: emilia.ricci2@unibo.it

D. M. Cordelli
Child Neurology and Psychiatry Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy

Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy
e-mail: ducciomaria.cordelli@unibo.it

F. Locatelli
Pathology Unit, Infermi Hospital, AUSL Romagna, Rimini, Italy
e-mail: francesca.locatelli@auslromagna.it

F. Caramelli
Pediatric Intensive Care Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy
e-mail: fabio.caramelli@aosp.bo.it

treatment (acyclovir), the outcome remains poor because of late identification of hepatic Herpes Simplex Virus (HSV) infection. We report a male neonate suspected with a metabolic/mitochondrial disease and multi-organ involvement but who developed a fulminant hepatic failure and disseminated coagulopathy secondary to HSV type 1 (HSV-1) infection. The postmortem diagnosis was performed demonstrating HSV-1 in liver tissue by transmission electron microscopy and by retrospective detection of HSV specific antigens by immunohistochemistry.

Keywords

Electron microscopy · Hepatitis · Herpes simplex · Postmorten diagnosis · Virus

1 Introduction

In the first 28 days-of-live intrauterine and perinatal infections are the major causes of neonatal death (Lehtonen et al. 2017). Neonatal Herpes Simplex Virus (HSV) infection is uncommon, deadly and caused by an enveloped virus of the Herpesviridae family (Basinger et al. 2019; Curry et al. 2006; Goldsmith 2014; Laue 2010; Motorna et al. 2018; Newcomb et al. 2017; Ramgopal et al. 2020; Roingeard 2008; Roingeard et al. 2019). HSV type 1 (HSV-1) causes 25–30% of the neonatal herpetic infections and leads to devastating diseases with potential poor outcomes. Infants acquire viruses during intrauterine, peripartum or postpartum periods, from a parent or caregivers. Neonates with HSV disease are classified in three groups: skin-eye-mouth, central nervous system (CNS), and disseminated disease. This classification is clinically relevant because it is predictive of neurodevelopmental outcome and mortality of the disease. Herpes Viruses, unlike Hepatitis Viruses, are not hepatotropic but may cause hepatitis, even if rarely (Noor et al. 2018). Most cases of HSV hepatitis rapidly progress to fulminant hepatic failure which can be fatal before both diagnosis and transplantation. Nowdays, although the pathogenesis of HSV hepatitis is not fully understood, it has high mortality rate and severe complications (Basinger et al. 2019; Noor et al. 2018; Then et al. 2019; Tosone et al. 2018). Clinical suspicion for neonatal HSV infection warrants immediate initiation of appropriate antiviral therapy with specific agents that dramatically improve clinical outcomes with manageable toxicity. For babies with disseminated and CNS disease, death is usually due to severe coagulopathy and extensive hepatic/pulmonary involvement despite the availability of therapy (McPherson 2020). This makes the early identification of HSV infection essential in improving outcomes and potentially preventing mortality (Then et al. 2019). We report a male neonate suspected with a metabolic/mitochondrial disease and multi-organ involvement who developed a fulminant hepatic failure and disseminated coagulopathy secondary to HSV-1 infection. The diagnosis was made postmortem demonstrating HSV in liver tissue by transmission electron microscopy (TEM) and detecting retrospectively HSV-1 specific antigens by immunohistochemistry (IHC).

2 Case Presentation

A male patient was born at term from dystocic delivery with the aid of vacuum extractor. Maternal pregnancy was normal with negative maternal serology (Cytomegalovirus, Toxoplasma gondii, HIV, Treponema pallidum, Rubella Virus, Hepatitis C Virus) and vagino-rectal swab (Streptococcus Agalactiae). He presented transient newborn respiratory distress responsive to nasal Continuous Positive Airway Pressure (nCPAP), a mild jaundice and a normal neonatal screening (Table 1) with negative Guthrie test, so he was discharged. On the third day of life, he was hospitalized at the Neonatology Unit (NU) of the birth point, due to hyperbilirubinemia (no laboratory data) and treated, until resolution, with phototherapy for 28 h. He was discharged on the fourth day of life in stable conditions and breastfed.

On the tenth day of life he was readmitted to hospital because of irritability, axial hypotonia, cyanosis and focal seizure activity and exposed to a broad-spectrum antibiotic therapy. In a few hours,

Table 1 Neonatal screening tests routinely done in the Neonatology and Neonatal Intensive Care Unit in S. Anna Hospital, Ferrara, Italy

Test	Result
Phenylketonuria	Negative
Hyperphenylalaninemia	Negative
Deficient biosynthesis of the biopterin cofactor	Negative
Deficient regeneration of the biopterin cofactor	Negative
Tyrosinemia type I and II	Negative
Maple syrup urine disease	Negative
Homocystinuria	Negative
Citrullinemia	Negative
Arginase deficiency	Negative
Argininosuccinate lyase deficiency	Negative
Carnitine transporter deficiency	Negative
Carnitine palmitoyltransferase I deficiency	Negative
Carnitine palmitoyltransferase II deficiency	Negative
Carnitine- acylcarnitine translocase	Negative
Very long chain acyl CoA dehydrogenase deficiency	Negative
Long chain 3 hydroxy acylCoA dehydrogenase and mitochondrial trifunctional protein deficiencies	Negative
Mediun chain acyl Coa dehydrogenase deficiency	Negative
Short chain 3 hydroxy acylCoA dehydrogenase deficiency	Negative
Glutaric aciduria type I	Negative
Glutaric aciduria type II	Negative
Isovaleric aciduria	Negative
Betaketothiolase deficiency	Negative
Propionic aciduria	Negative
Methylmalonic aciduria	Negative
Methylmalonic aciduria with Homocystinuria	Negative
3Hydroxy 3 methyl glutaric aciduria	Negative
2 Methylbutirril CoA dehydrogenase deficiency	Negative
Multiple carboxilase deficiency	Negative
Galactosemia	Negative
Biotinidase deficiency	Negative
Congenital adrenal hyperplasia	Negative
Congenital hypothyroidism	Negative
Cystic fibrosis	Negative

his clinical conditions dramatically worsened with impaired respiratory and cardiac function and oliguria. Laboratory tests showed: metabolic acidosis, coagulopathy, hyperammonemia, hypertransaminasemia, ketotic hypoglycemia (Table 2). The patient was transferred at our Pediatric Intensive Care Unit (PICU) where he arrived in profoundly serious conditions (Table 2); the PEdiatric Logistic Organ Dysfunction (PELOD) score was critical equal to 22.7. Supportive care was immediately started with metabolic correction measures, mechanical ventilation, inotrope (VIS first 24 h = 60), peritoneal dialysis after insertion of Tenckhoff catheter and blood products transfusion. Plasma amino acidogram showed a generalized elevation of all amino acids and plasma acylcarnitines determination showed minimal elevation of C2-C4-C8-C10. Hepatotropic virus (Epstein Barr Virus, Parvovirus, Cytomegalovirus) serology was negative. However, all treatments proved ineffective and the patient died 48 h after admission. Muscle and skin biopsies and DNA sample were taken in perimortem, with informed consent from parents, as usual in children with metabolic disease with not defined etiology. Autopsy was then performed.

Table 2 Laboratory data from the tenth to the twelfth day of life of the patient

	Age of life			
Exam	10th day (NU, Ferrara)	10th day (PICU, Bologna)	11th day	12th day
AST (<60 U/L)	–	7,662	4,647	–
ALT (<45 U/L)	2,714	1,254	729	–
PT (<1.20 s)	Sample does not coagulate	Sample coagulated	5.90	Hemolysis
A PTT (0.82–1.25 s)	Sample does not coagulate	Sample coagulated	7.8	Hemolysis
pH	–	7.15	7.14	6.91
LACTATE (0.5–1.4 mmol/L)	–	>15	>15	>15
BE (48 mmol/L)	–	−19.2	−13.8	−24.3
AMMONIA (<53 μmoli/L)	621	335	248	–
PCR (<0.50 mg/dl)	0.29	0.39	0.19	–
GLUCOSE (45–126 mg/dl)	<10 (ketons in urine)	–	–	–

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *PT* prothrombin time, *APTT* partial thromboplastin time activated, *BE* base excess, *PCR* C-reactive protein

2.1 Muscle Biopsy

Muscle biopsy performed 1 day after hospitalization was snap-frozen in liquid nitrogen-chilled isopentane. Cryostat-cut sections were routinely stained for routine histology and histoenzimology and small fragments of muscle biopsy were fixed and embedded in araldite for analysis under TEM, as previously described (Cenacchi et al. 2013). Histological and histochemical stainings showed myopathic, aspecific findings such as fiber size variability, rare hypertrophic eosinophilic fibers, and slightly increased staining in the reaction for lysosomal acid phosphatase. Ultrastructural study showed few alterations of the myofibrillar component with disarray and mild increase of lipids with no marked evidence of mitochondria alterations. The histological and ultrastructural findings didn't confirm a diagnosis of mitochondrial or metabolic disease.

2.2 Autopsy

On autopsy, gross examination revealed multi organ failure with anasarca, pulmonary edema, bilateral cortical kidney ischemia, bilateral adrenal sub-atrophy and diffuse liver damage evidenced by small white areas in a red background, suggestive of necrosis caused by infection and/or shock. The cause of hepatic damage was not obvious at the time, but it was thought to be the initial cause of a sequence of damaging events. As routinely done, a thorough sampling of the internal organs was performed for histological study. Liver samples were submitted also to ultrastructural analysis (UA) because of the clinical suspicion of mitochondrial or metabolic disease and pathological suspicion of infectious disease. Moreover, hemorrhagic subcutaneous suffusions were suspicious for hepatic insufficiency and/or intravascular coagulopathy. Small gastric ulcers associated with hemorrhagic punctuations of the mucosa and mild splenomegaly were also described. Brain examination was not performed for ethical reasons.

2.3 Liver and Adrenal Gland Histology Analysis

Autoptic liver and adrenal gland biopsies were formalin fixed, paraffin embedded and stained with HE which showed a severe necrotizing hepatitis with confluent hemorrhagic necrosis with few areas of coagulation necrosis (Fig. 1a, b). Hepatic hemorrhagic necrosis is also a consequence of the shock associated with disseminated intravascular coagulopathy. Adrenal gland showed small areas of coagulation necrosis (Fig. 1c, d), that might have contributed to organ failure shock.

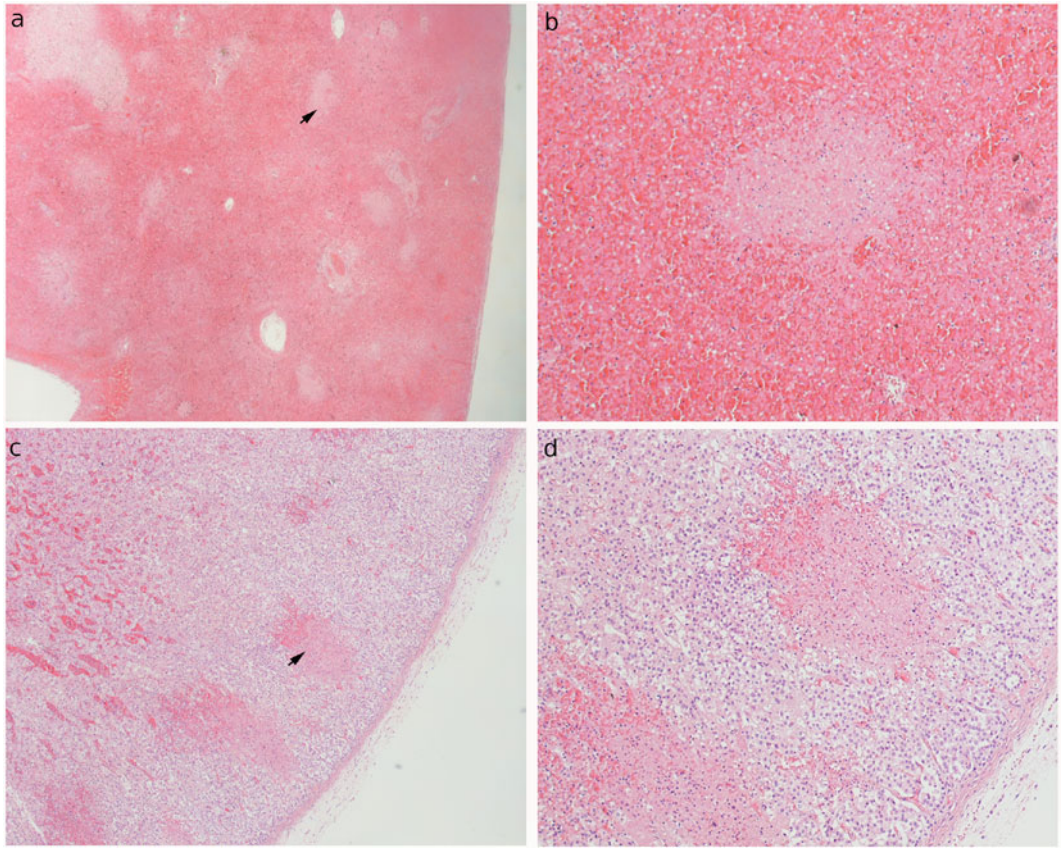

Fig. 1 Liver and adrenal gland autoptic samples, histology analysis. HE shows several areas of coagulation necrosis (arrows) in liver (**a**, **b**) and adrenal gland (**c**, **d**); magnification 2x (**a**, **c**) and 20x (**b**, **d**)

2.4 Liver Ultrastructural Analysis

As previously described small liver fragments were processed for UA, which showed hepatocytes with intracytoplasmic large lipid vacuoles (Fig. 2a) and irregularly shaped nuclei, despite artefactual post-mortem alterations (Cenacchi et al. 2013). Moreover, nucleoplasm and cytoplasm featured many roundish or polygonal nucleocapsid particles, 70–80 nm in diameter with an electron dense core (Fig. 2b). These particles were also found near the nuclear membrane frequently empty (defective particles) (Fig. 2c). In the intercellular areas some viral nucleocapsids were seen showing an envelope with an overall diameter of about 200 nm (Fig. 2d).

2.5 Liver and Adrenal Gland Immunohistochemical Analysis

After the result of UA, IHC was performed using a cocktail of anti-HSV antibodies composed by a rabbit anti-HSV-1 and a mouse anti-HSV-2; this cocktail reacts with both HSV-1 and HSV-2 specific antigens and it recognizes all the major glycoproteins in the viral envelope and at least one core protein (PDRM001, Diagnostic Biosystem). Using different secondary antibodies, respectively anti-rabbit and anti-mouse separately on different slides, we were able to identify nuclei and some cytoplasmic areas positive for HSV-1 and negative for HSV-2 both in liver (Fig. 2e) and adrenal gland (Fig. 2f). The overall histological

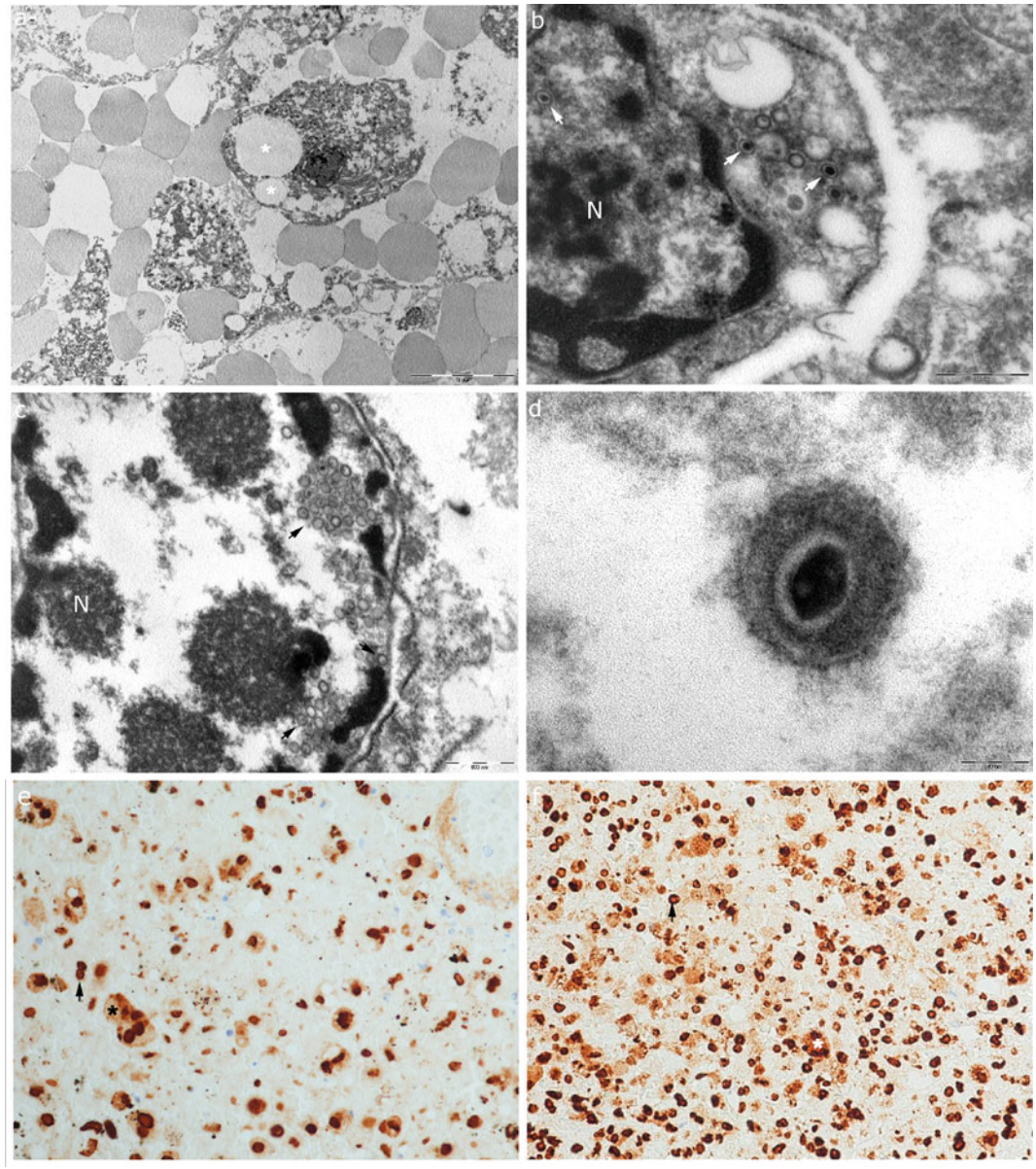

Fig. 2 Liver and adrenal samples, ultrastructural and IHC analysis. (**a**) Remnants hepatocytes with cytoplasmic lipid vacuoles (asterisks). (**b**) Nucleocapsidic particles with electrondense core are visible in the nuclei and cytoplasm (arrows). (**c**) Some empty defective particles are found alongside the nuclear membrane (arrows). (**d**) In the intercellular space, some virions are visible with an envelope of intermediate electrondensity, giving an overall diameter of 200 nm. Numerous HSV-1 positive nuclei in hepatic (**e**, arrow) and adrenal (**f**, arrow) tissues and few cytoplasmic areas (asterisks) by IHC analysis. Magnification 5,400x (**a**), 25,000x (**b**, **c**), 64,000x (**d**), 40X (**e**, **f**)

findings suggest an hematogenous dissemination of the HSV infection.

3 Discussion

Neonatal herpetic infections should be considered in the differential diagnosis of each acutely unwell neonate. Moreover, disseminated HSV infection should always be considered as a possibility, especially in neonates with CNS, hepatic, pulmonary or multiorgan disease processes. Early diagnosis is imperative when evaluating neonatal HSV infection to prevent further disease progression, neurological complications and even death. Even though treatment (acyclovir) is readily available, most cases have a poor prognosis due to late therapeutical initiation because of an infection misdiagnosis (Abuhasna et al. 2012). The differential diagnoses to consider in disseminated disease patients include other viral causes and metabolic/mitochondrial diseases. HSV infection might be suspected even though its presentation is frequently non-specific, difficult to be distinguished from bacterial sepsis and the maternal infection can be asymptomatic. Diagnostic tools include cultures of vesicular lesions and 'surface swabs', HSV serology, HSV DNA by PCR and liver biopsy histological examination. The latter remains the gold standard for diagnosing HSV hepatitis even if there is an increased associated risk of bleeding in patients with acute liver failure, sometimes reduced by a transjugular approach instead of a percutaneous one (Noor et al. 2018; Then et al. 2019). In the literature, this very rare diagnosis is frequently made through post-mortem biopsy, confirming that HSV hepatitis is usually characterized by a non-specific presentation, making timely diagnosis difficult and fatally delaying the following treatment. In our case, we have considered but excluded neonatal sepsis due to repeatedly negative C-reactive protein (Table 2) and supposed a potential mitochondrial/metabolic disease because of the early onset of symptoms, elevated ammonia and lactate as well as metabolic acidosis and ketotic hypoglycemia found already from onset. Histological and ultrastructural findings of the muscle biopsy did not confirm this hypothesis. We tried to investigate primary hepatic diseases, also infective ones (Epstein Barr, Parvovirus, Cytomegalovirus) but they were all negative. The extremely serious conditions of the child made difficult even recovering blood samples on which perform further analysis and the research of HSV DNA, which was possible only on DNA isolated from Guthrie test, was negative as well, excluding a peripartum maternal infection. We managed to carry out the plasma amino acidogram, which showed nonspecific findings, probably influenced by severe hemolysis, and the determination of plasma acylcarntins which did not justify the overall clinical picture. These no specific results couldn't guide appropriate genetic tests. The UA of the liver biopsy suspected the presence of enveloped viral particles compatible with Herpes Virus. Herpes and Cytomegalovirus share a similar ultrastructural morphology and the differential diagnosis has been done by IHC analysis. In addition, in the histology findings of the liver biopsy were absent cytomegaly and enlarged nuclei with the "Owl's eye" inclusions, typical of a Cytomegalovirus infection. IHC on liver sections identified HSV-1 as the causative agent, confirming the ultrastructural suspicion and reversing the initial metabolic hypothesis, which would have been negative for the parental perspective of future sons. In conclusion, we would like to aid clinicians to early identify of HSV hepatitis to rapidly initiate treatment. The antiviral therapy prevent mortality even if we cannot forget that liver transplantation may be the only option for neonates with fulminant or acyclovir resistant HSV hepatitis (Noor et al. 2018; Shahani 2016; Then et al. 2019; Vincenzi et al. 2017). Moreover, we would like to highlight the key role of the UA in viral infection diagnosis also in autoptic samples.

Declarations

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Ethics Approval Written informed consent for muscle and skin biopsies and autopsy were obtained from the patient's parents.

Availability of Data and Material Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Code Availability Not applicable.

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