Journal of Clinical Virology 58 (2013) 486–489

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Case report

Post-mortem diagnosis, of cytomegalovirus and varicella zoster virus co-infection by combined histology and tissue molecular biology, in a sudden unexplained infant death

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ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 23 July 2013
Accepted 5 August 2013

Keywords:
Sudden unexpected infant death
Virology
Histopathology
Real-time PCR assay
Cytomegalovirus
Varicella zoster virus

ABSTRACT

Background: An autopsy case of a two-month-old male infant who suddenly and unexpectedly died during his sleep, eight days after the onset of benign varicella.
Objectives: To describe post-mortem combined histological and tissue molecular biological techniques for the diagnosis of cytomegalovirus and varicella zoster virus co-infection as a cause of death.
Study design: Real-time quantitative PCR and RT-PCR assays for Herpesviruses, respiratory viruses, Adenovirus, Enterovirus and Parvovirus B19 were performed on multi-organ frozen samples and paraffin-embedded tissues in combination with histology.
Results: Cytomegalovirus and varicella zoster virus were detected by molecular biology with highest viral loads detected in the lungs (4.6×10^7 and 1.9×10^5 genome copies per million of cells, respectively). Pulmonary extensive necrotizing inflammation and immunohistochemistry correlated to virological data. Virological molecular biology was negative on paraffin-embedded tissues.
Conclusions: This case shows that thorough quantitative virological investigations on frozen tissues must be performed in combination with histology and immunohistochemistry for the determination of the cause of a sudden unexplained infant death.

1. Why this case is important

Previous studies have demonstrated the major role of viral diseases in the pathogenesis of sudden unexpected infant death (SUID) [1–4]. We report on a case of a two-month-old male infant who suddenly and unexpectedly died during his sleep, without premonitory symptoms. Post-mortem virological quantitative molecular analyses performed on frozen tissue samples revealed cytomegalovirus (CMV) and varicella zoster virus (VZV), mainly in the lungs. These results correlated to histological findings. Virological molecular biology was negative on paraffin-embedded tissues.

This case shows that quantitative virological investigations on multi-organ frozen samples must be performed in SUID in combination with histology and immunohistochemistry. Without this diagnostic approach, viral infections can be underestimated as a cause of death in infants.

2. Case description

A two-month-old male infant was found dead in his cot. According to national diagnostic protocol in SUID, an autopsy was performed in our institution.

His mother had positive VZV and CMV serologies with antibodies titers of, respectively, 1800 international units (IU) and 560 arbitrary units (AU) per liter (Enzygnost® assay, Siemens, Erlangen, Germany) associated with high anti-CMV IgG avidity during pregnancy, suggestive of a maternal primary infection before pregnancy. The infant was prematurely delivered at thirty-six weeks. The newborn was hospitalized during the first sixteen days after delivery in a neonatal intensive care unit, because of a severe

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http://dx.doi.org/10.1016/j.jcv.2013.08.007

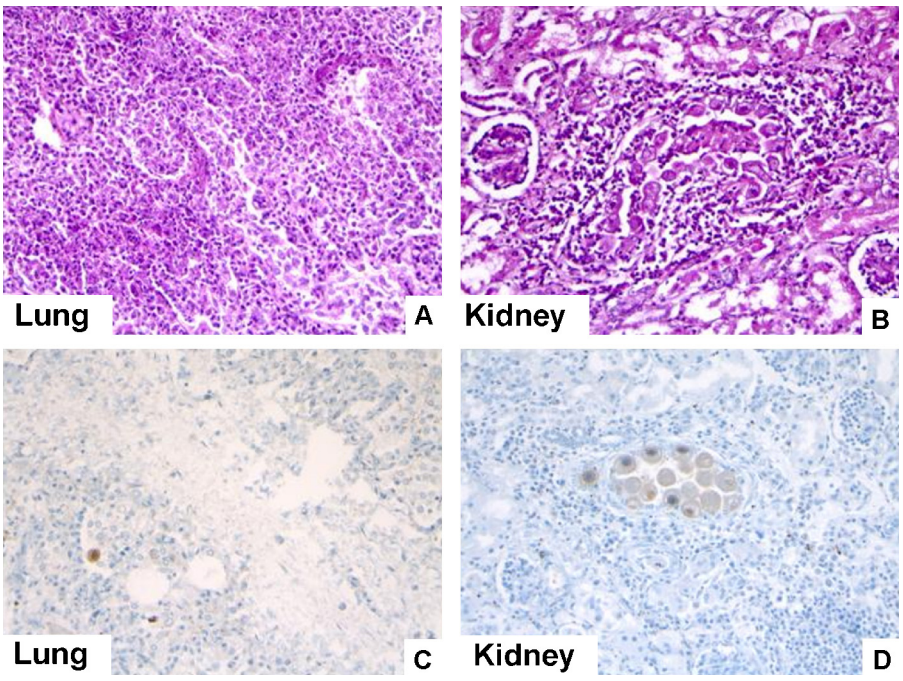

Fig. 1. Pulmonary (A and C) and renal (B and D) histological and immunohistochemical features. (A) Hematoxylin eosin-stained pulmonary sections showing necrotizing inflammation (×200). (B) Hematoxylin eosin-stained renal inflammation with moderate mononuclear cell infiltrates (×200). (C) A CMV cell inclusion detected in lung by anti-CMV anti-body (brown stained cell; ×200). (D) Numerous CMV inclusions detected in renal tubular cells by anti-CMV antibody (brown stained cells; ×200).

hypotrophy (weight: 1725 g) associated with thrombopenia-related diffuse petechiae (58,000/mm^3 platelets). Platelet count increased spontaneously, up to 148,000/mm^3, with no explanation. No perinatal investigations of CMV congenital infection were performed. The newborn weighted 2080 g when he was discharged.

At the age of one month and 21 days, he had varicella contracted from his brother who had been diagnosed with chickenpox 15 days earlier. The newborn's infection was mild, consisting of three successive skin rashes, with no other manifestations. No antiviral treatment was given. Eight days after the onset of the varicella, he was found dead on the back in his cot. In this context of a SUID, an autopsy was performed according to national protocol. His weight was 3725 g. Disseminated skin necrotic lesions were present. The organs were found normal. No cause of death was found. Viral (Herpes Simplex virus type 1 and type 2 (HSV-1 and -2), CMV, Epstein–Barr virus (EBV), VZV, Human Herpes Virus type 6 (HHV-6) and Enterovirus) and bacterial tests were negative in the cerebrospinal fluid (CSF). Interferon alpha was negative in the CSF. Viral cultures performed from skin lesions, throat and anal swabs, as well as from tracheobronchial aspiration were negative. Hemocultures, throat and fecal cultures were negative for main bacterial pathogens.

Histology showed extensive necrotizing inflammation in the lungs and moderate mononuclear inflammation in the kidneys (Fig. 1A and B). Other organs were otherwise normal. Immunohistochemistry on paraffin blocks using monoclonal CMV antibody (Argene bioMerieux, Verniolle, France) revealed a few CMV cell inclusions in the lungs whereas numerous CMV inclusions were observed in the kidneys (Fig. 1C and D). Heart, lung, kidney, liver, small intestine and colonic samples were collected for virological analyses and stored at −80 °C. These samples were analyzed with real-time PCR and RT-PCR assays for herpesviruses (HSV-1, HSV-2, VZV, CMV, EBV and HHV-6), respiratory viruses (Influenza A&B viruses, Parainfluenza 1 to 4 viruses, Respiratory Syncytial Virus (RSV), Rhinovirus, human Bocavirus (hBoV), human Coronavirus NL63, OC43, HKU1, 229E and the human Metapneumovirus), Adenovirus, Enterovirus and Parvovirus B19 (HSV1 HSV2 VZV r-gene®, Respiratory Multi Well System r-gene®, Argene bioMerieux, Verniolle, France) [5–10]. CMV and VZV genomes were detected in all tissue samples (Fig. 2). The highest CMV and VZV loads were found in the lungs, 4.6×10^7 and 1.9×10^5 genome copies per million of cells, respectively (Fig. 2). In contrast, CMV and VZV DNA were not detected in paraffin-embedded tissues.

3. Other similar and contrasting cases in the literature

Several studies have demonstrated frequent detection of viruses in post-mortem specimens suggesting their involvement in the pathogenesis of SUID [1–4]. The main viruses reported were respiratory viruses, such as RSV, Adenovirus, Influenza and Parainfluenza viruses, responsible for respiratory tract inflammation, apnoea and hypoxemia, which can exceptionally lead to death [2,3,11.–18]. Other viruses involved in common childhood diseases such as HSV, EBV, CMV, HHV-6, Parvovirus B19 and Enterovirus have also been detected in lungs, heart and CSF of deceased infants,

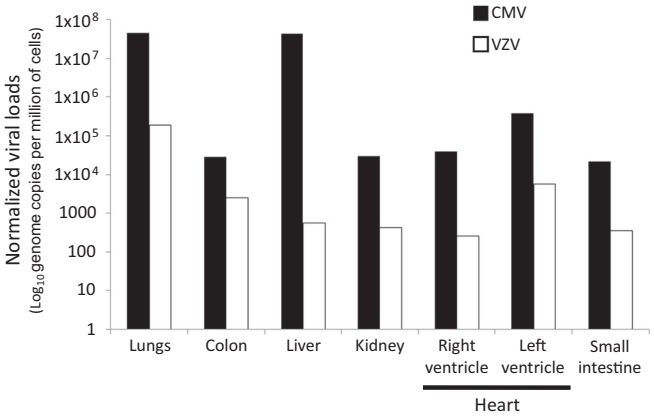

Fig. 2. CMV and VZV loads, normalized per million of cells, assessed in multi-organ frozen tissues sampled during the autopsy.