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CASE REPORT

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Epstein–Barr virus myocarditis as a cause of sudden death: two autopsy cases

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Abstract Although the Epstein–Barr virus (EBV) causes acute infection accompanied by a high fever in young people, there appears to be few reports of a fatal outcome involving myocarditis. We report two cases of unexpected sudden death due to acute myocarditis possibly caused by the EBV. They each visited a hospital due to common cold-like symptoms and unexpectedly died several days later. In both cases, autopsy revealed myocardial necrosis with marked lymphocytic infiltration. Polymerase chain reaction (PCR) screening was positive for the EBV, whereas immunohistochemistry and in situ hybridization for the EBV were negative. Serological investigations showed a mild elevation in antiviral capsid antigen IgG and anti-EBV nuclear antigen IgG in both cases. Immunohistochemical study of lymphocytic infiltrates showed strong positivity for a T-cell marker ($CD45R_0$) in the myocardium and pharyngeal mucosa. These cases suggest the potential risk of mortality from acute EBV infection in young people, even without severe clinical manifestations, and the importance of microbiological investigations, including PCR procedures, in postmortem diagnosis of infectious diseases.

Keywords Forensic pathology · Myocarditis · Epstein–Barr virus · Immunohistochemistry · Polymerase chain reaction

Introduction

Myocarditis is an important cause of unexpected sudden death in young people [1, 2]. Myocarditis is defined pathologically as "inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes not typical of the ischemic damage associated with coronary artery disease" [3]. Its clinical diagnosis may be difficult due to subclinical or nonspecific signs and symptoms. Although it has various etiologies, viral myocarditis is the most frequent [4–6]. Approximately half of all cases have been attributed to the Coxsackie B virus, and most of the remainder to Coxsackie A, echovirus, and poliovirus [7]. The Epstein–Barr virus (EBV) is rarely involved, and there seems to be few reports of a fatal case [8–10].

The EBV is a member of the Herpesviridae family. It is a ubiquitous virus that infects most people. The EBV mainly affects B lymphocytes via the EBV receptor on the cell surface, which are eliminated by EBV-antigen-directed T lymphocytes. EBV is also an oncogenic virus, which causes malignancies such as lymphomas and nasopharyngeal carcinoma. Although serological and molecular biological procedures have been established for clinical diagnosis of EBV infection, identification of the viral infection is difficult in the early phase.

We describe two unexpected deaths of young people due to acute myocarditis, in which EBV was identified by a polymerase chain reaction (PCR) assay, and discuss the medicolegal importance.

Case 1

Case history

The deceased was a 17-year-old young woman. She had cold-like symptoms with a high fever for several days. Although she took a non-prescription over-the-counter drug containing aspirin, the symptoms persisted. She visited a local hospital, and medication was prescribed. However, her symptoms did not improve and she again visited the hospital on the following day. Just after an X-ray examination, she lost consciousness and fell down on the floor. Despite cardiopulmonary resuscitation, including electrical defibrillation and intravenous injection of adrenalin, the

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heartbeat was not recovered. The cause of death was clinically undetermined. An autopsy was performed about 30 h postmortem because of suspected medical malpractice.

Autopsy findings

The body, 169 cm tall and weighing 61 kg, was pale, with a dark, reddish-purple hypostasis on the back. The palpebral conjunctivae were edematous with no petechiae. In the pericardial cavity, there was ca. 30 ml of a yellowish clear effusion. The heart weighed 295 g, with numerous petechial hemorrhages in the lower posterior wall and enlarged chambers, which were filled with blood containing soft clottings. A small mural thrombus was attached at the apex of the left ventricle, where multifocal necroses were seen (Fig. 1). The lungs (left, 500 g; right, 620 g) were congested and edematous, showing multiple ecchymoses on the cut surface, with small amounts of pleural effusion. The liver (1,580 g) had a smooth surface and was markedly congested. The spleen (355 g) was enlarged. Although the pharyngeal lymph nodes were enlarged, there was no systemic lymph node enlargement. There was no evidence of other pathology or trauma.

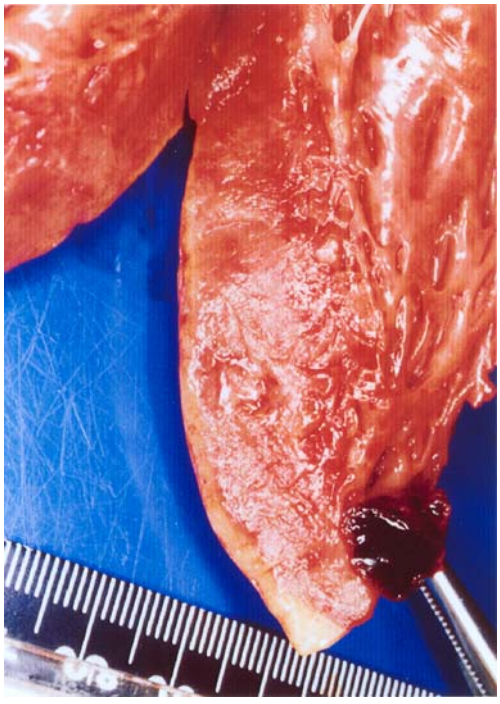

Fig. 1 Multifocal necroses are seen at the apex of the myocardium. The lesions were independent of the vascular bed, having unclear margins, and evident in the epicardial region of the myocardium. Mural thrombi were found in the cardiac apex, possibly due to blood stasis resulting from the left venticular wall motion abnormality and hypercoagulability because inflammation may have played an important role in the thrombogenesis

Case 2

Case history

A 24-year-old man had visited a hospital several days before his death, suffering a high fever (38°C), coughing, and epigastragia. The physician suspected infectious gastritis and prescribed medication. Three days later, because of his absence from the workplace without leave, his colleague visited his home and found him dead, lying supine on a mattress. An autopsy was performed because the cause of death was unknown.

Autopsy findings

The body, 170 cm tall and weighing 63 kg, was pale, showing marked dark reddish-purple hypostasis on the back. The palpebral conjunctivae were mildly congested without petechiae. There was ca. 50 ml of a turbid pericardial effusion with mild hemoglobin imbibition. The heart weighed 490 g, with many petechial hemorrhages on the posterior wall and enlarged chambers containing a large amount of clotting. Colliquative necrosis was observed in the myocardium at the posterior region of the septum. The lungs (left, 490 g; right, 640 g) were congested and edematous, showing multiple ecchymoses on the cut surface, with small amounts of plural effusion. The liver (1,700 g) had a smooth surface, and was markedly congested. The spleen (160 g) was congested. There was no evidence of other pathology or trauma.

Laboratory investigations

Histology

In cases 1 and 2, the cardiac histopathology was quite similar as follows: intense inflammatory infiltrates, composed of lymphocytes, histiocytes, and eosinophils, were observed in the interstitia of both ventricles, involving the conduction system. Focal myocardial necrosis was seen (Fig. 2). In the pharyngeal mucosae, hemorrhages and inflammatory cell infiltration were observed. There was mild lymphocyte infiltration in the Glisson's capsules without involvement of hepatocytes in the liver. Azan staining showed mild proliferation of fibrous components in the hemorrhagic areas of the myocardial interstitium and pharyngeal submucosal tissue.

Immunohistochemistry and in situ hybridization

To detect EBV-specific proteins in paraffin-embedded tissues, immunostaining using monoclonal antibodies against latent membrane protein (LMP)-1 and EBV nuclear antigen (EBNA)-1 (Novocastra, Newcastle upon Tyne) were performed. The antibodies (1:50 diluted) were incubated overnight at room temperature and were visualized with a streptavidin–biotin–horseradish peroxidase complex and the

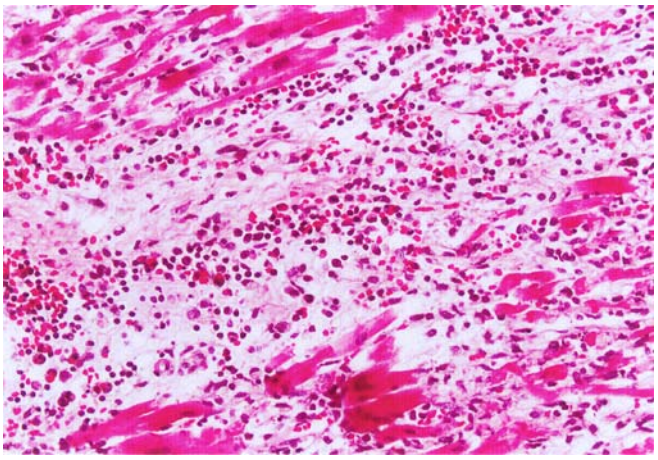

Fig. 2 Photomicrograph of HE-stained section of myocardial tissue in case 1. Active inflammatory infiltrates consisted of lymphocytes, histiocytes and eosinophils separates the cardiac myofibers. Necrosis is evident. (magnification ×200)

3,3′-diaminobenzidine/hydrogen peroxide staining method, as described previously [11]. EBV-positive paraffin-embedded liver tissue sections were used for positive control. In cases 1 and 2, immunostaining of LMP-1 and EBNA-1 for detection of EBV showed no positive reaction in myocardial and pharyngeal tissues. In situ hybridization for the EBV was also negative. The lymphocytes in the inflammatory sites in the myocardium and pharyngeal mucosa showed strong positivity for a T-cell marker ($CD45R_0$). Only a few B cell marker (CD20)-positive lymphocytes were found (Fig. 3).

Postmortem microbiology

Unfixed myocardial tissues were examined by means of PCR to detect the adenovirus, measles virus, influenza virus, herpes simplex, cytomegalovirus, EBV, and hepatitis C virus. The conventional PCR primer sequences for detection of seven viruses were designed in accordance with the cording sequences of the structural proteins of the viruses. For EBV, 5′-TCCTCGTCCAGCAAGAAGAG-3′ for the forward primer and 5′-CAACTTGAGGCAGCC TAATCC-3′ for the reverse primer were used. The expected sizes of the amplified sequences in the EBV genomes were 161 base pairs. PCR amplification conditions were as follows: 40 cycles of amplification including denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and primer extension at 72°C for 2 min. The PCR showed positive findings only for the EBV DNA. PCR investigations of the spiral fluid and pericardial effusion gave negative findings.

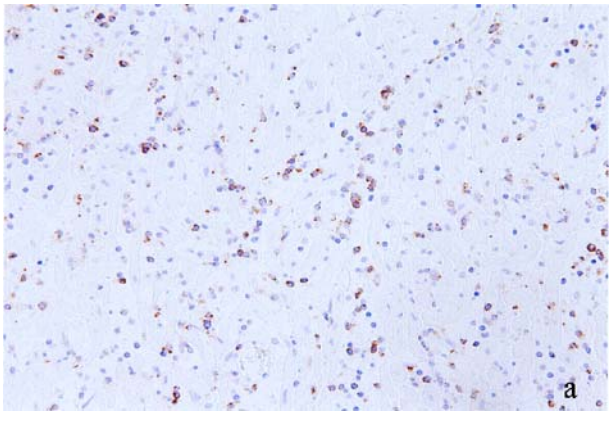

Fig. 3 Infiltrated lymphoid cells are predominantly $CD45R_0$-positive cells in case 1 (magnification ×400)

Table 1 Postmortem laboratory findings

	Case 1	Case 2	Reference interval
Serum blood chemistry (right ventricle)			
IgG	1210	905	900–1,700 mg/dl
IgA	114	245	140–300 mg/dl
IgM	130	40	70–170 mg/dl
IgD	<0.6	3.0	3 mg/dl
C-reactive protein	7.69	5.11	<0.19 mg/dl
Neopterin	662	196	2–8 pmol/ml
IL-4	111	21.5	<6.0 pg/ml
Pericardial fluid			
HANP	25	<10	<40 pg/ml
BNP	256	13.8	<20 pg/ml

Table 2 Results of the serological investigations with immunoglobulin

			Case 1	Case 2	Reference value
EBV	anti-EA	IgG	0.3	0.3	<1.0
EBV	anti-VCA	IgG	8.9	7.5	<1.0
EBV	anti-VCA	IgM	0.0	0.3	<1.0
EBV	anti-EBNA	IgG	6.6	13.3	<1.0
EBV	anti-VCA	IgA	<10	<10	<10
EBV	anti-EA-DR	IgA	<10	<10	<10

Postmortem biochemistry and serological tests

There was a moderate elevation in serum C-reactive protein and an elevation in serum neopterin and cytokine IL-4 (Table 1). Serum immunoglobulins were within clinical reference intervals. For the EBV, anti-viral capsid antigen (VCA) IgG and anti-EBV nuclear antigen (EBNA) IgG showed mild elevation (Table 2). The antibody titers of the other viruses were not elevated.

Discussion

In the present two cases, macroscopic findings of myocardial colliquative necrosis and "histopathology implying injury to myocardial fiber adjacent to the infiltration site of inflammatory cells"[3] were compatible with the diagnostic criteria of myocarditis. There was no pathological evidence for other diseases including effusion lymphoma [12–14] or

EBV hepatitis; there were no significant amounts of pleural effusion, systemic lymphadenopathy or active inflammation in the liver. Furthermore, histological findings and an elevation in serum neopterin strongly suggested a viral etiology, which was also consistent with the clinical symptoms involving a persistent high fever. Serum neopterin level is known to increase during the acute phase of inflammatory diseases and reflect disease severity [15]. In the clinical diagnosis of viral infections, serological and microbiological investigations are combined. In particular, identification of a virus at the site of inflammation is a clue for a diagnosis of viral infection. For this purpose, molecular biological procedures using PCR can be used in clinical and postmortem investigation [16, 17]. In the present cases, the EBV was identified at the site of myocarditis. A mild elevation in the serum antibodies to the virus, as discussed below, was also observed. B lymphocytes, which have been transformed by EBV infection, may contribute to the myocardial damage at the site of inflammation. However, in the present cases, there was a predominance of T-cell marker ($CD45R_0$)-positive lymphocytes in the inflammatory sites in the myocardium and pharyngeal mucosa, and an elevation was observed in serum IL-4 level, possibly derived from T cells [18, 19]. These findings were different from chronic active EBV infection, in which B cells are increasing [20]. Although T cells usually play an important role in controlling viral infections, they may have mainly contributed to myocardial injury. There were some reports that T cells are involved in the pathology of myocarditis due to chronic EBV infection [21–23]. Otherwise, B cells affected by the EBV may have been scavenged by T cells [24].

Negative findings in in situ hybridization and immunohistochemistry may be attributed to the limited sensitivity. In this respect, Morey et al. [25] reported a decrease in positivity in the immunohistochemical method for virus identification in formalin-fixed paraffin-embedded specimens. Takeuchi et al. [17] and Tierney et al. [26] reported that the EBV could not be detected by in situ hybridization in cases of EBV infection. PCR procedures are the most sensitive and potentially useful for identification of viruses in postmortem investigations [27], when used in combination with histological and serological methods, incorporating adequate control materials. In this procedure, EBV genomes from a latent infection may be incidentally detected due to a very high sensitivity. In addition, at present, PCR procedures have not been established for some other viruses, thus raising difficulty in excluding other viral infections. However, in the present cases, acute EBV infection was considered to be the possible cause of myocarditis in combination with the macro-, micropathological, and serological findings.

It is known that compared with identification of a virus from feces or fluid swabs from the throat, serological investigations of serum antibody titers provide poor evidence for diagnosis of viral infections, especially in the acute phase [28]. The positive incidence of serum virus antibody titers in acute myocarditis was as low as 28.2%, and a negative finding may not exclude viral etiology [29]. Kawa [30] reported that serum VCA IgM and early antigen (EA)-DR IgG antibodies showed an elevation level several weeks after infection, followed by VCA IgG and then EBV nuclear antigen (EBNA) antibodies, which appeared about 2 months later. In the present two cases, pathological findings suggesting an early inflammatory phase possibly about 5 days postinfection were compatible with a significant but mild elevation in the antibody titer, despite normal immunoglobulin levels. There are various viruses that cause myocarditis, although picornavirus is the most frequent. More than half of myocarditis cases are due to the Coxsackie virus, with the remainder due to viruses such as echovirus and poliovirus [31]. Viral myocarditis is not rare, although a rapid fatal outcome such as observed in the present cases may be infrequent. In the clinical diagnosis of myocarditis, chest X-rays, electrocardiography (ECG), serum myocardial markers, serum antibody titers, ultrasonography, myocardial scintigraphy, and myocardial biopsy are used. However, the incidence of positive viral antibodies in acute myocarditis is low [29], as mentioned above. In addition, varied incidences (9–78%) for the efficacy of myocardial biopsy have been reported [29, 32]. Meanwhile, it is reported that ECG is the most useful, showing abnormal findings in 97% of cases even in the acute phase [31]. In the present cases, possibly due to common cold-like symptoms, ECG was not performed. Viral myocarditis is a very serious disease with a high acute-phase mortality. However, a good prognosis may be expected when intensive clinical care and management are undertaken after an early diagnosis in the acute phase.

Acute EBV infection is not rare in young people, usually causing a persistent high fever. However, two cases of unexpected death due to acute myocarditis, possibly caused by the EBV, as described in this report, strongly suggest a potential risk of mortality in acute EBV infection. Careful management, including ECG examination, is recommended.

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