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PCR-based diagnosis of cytomegaloviruses in paraffin-embedded heart tissue in cases of suspected sudden infant death syndrome (SIDS)

R. Dettmeyer *, J. Müller, S. Poster, B. Madea

Institute of Forensic Medicine, University of Bonn, Stiftsplatz 12, 53111 Bonn, Germany

Abstract. Immunohistochemical and molecular-pathological techniques have improved the diagnosis, but the incidence of virus-induced lethal myocarditis remains unclear. Therefore, it is of great interest to investigate postmortem myocardial samples in cases of sudden infant death syndrome (SIDS). Cytomegaloviruses are known as possible agents of myocarditis. Viral DNA was specifically isolated and amplified from formaldehyde-fixed material. At autopsy, myocardial samples were taken from 70 SIDS cases from different regions and investigated with PCR. Cytomegalovirus DNA was detected in 2 out of 70 cases. Our results emphasize the importance of modern molecular-pathological methods in cases of sudden unexpected death.

Keywords: Cytomegaloviruses; PCR; Paraffin-embedded tissue; Myocarditis

1. Introduction

Studies of myocarditis in adults demonstrated that myocarditis often cannot be diagnosed, according to the Dallas criteria [1]. Previously, we reported on detection of enteroviruses (EV) including coxsackieviruses B3 (CVB3), parvovirus B19 (PVB19), adenoviruses (AV) and Epstein-Barr virus (EBV) [2–6]. Cytomegaloviruses belong to the herpesviridae family and have occasionally been associated with myocarditis. We analysed cytomegalovirus DNA from paraffin-embedded heart tissue with PCR.

2. Materials and methods

Postmortem myocardial samples were obtained from 70 autopsy cases with suspected sudden infant death syndrome (SIDS). Eight myocardial samples were taken from each

* Corresponding author. Tel.: +49 228 73 8331; fax:+49 228 73 8339.
E-mail address: rdettmey@uni-bonn.de (R. Dettmeyer).

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Table 1
Details of PCR primers used to amplify viral genes; CMV; cyc

Primer	Nucleotide sequences (5′ to 3′)	Product size (bp)
CMV 1	CCA AGC GGC CTC TGA TAA CCA AGC C	435 bp (CMV 1/CMV 2; first round)
CMV 2	CAG CAC CAT CCT CCT CTT CCT CTG G	
CMV 3	AGT GTG GAT GAC CTA CGG GCC ATC G	110 bp (CMV 3/CMV 4; second round)
CMV 4	GGT GAC ACC AGA GAA TCA GAG GAG C	
CycF	CGTC CAG CAT TTG CCA TGG A	180 bp
CycR	GAC AAG GTC CCA AAG ACA G	

heart at standardized locations. Viral DNA was extracted from paraffin-embedded myocardial, liver and spleen samples with the Genial First-DNA-Kit (Genial, Troisdorf, Germany). The prerequisite for virus PCR was the amplification of cyclophilin (cyc) [7]. To avoid false-positive results, negative controls were performed in all experiments [8]. For each assay, positive controls were added. For details of PCR, see Tables 1 and 2.

PCR products were sequenced on an ABI 310 sequencer. Sequence comparison was performed by BLAST search of NCBI Gen-Bank. PCR-products were also analysed on polyacrylamide gels (Fig. 1).

3. Results

Cytomegalovirus DNA was detected in 2 out of 70 cases of suspected SIDS. In 1 of these 2 cases, the myocardial samples also revealed one single minimal focus with signs of myocarditis according to the Dallas criteria using conventional histologic stainings.

4. Discussion

Acute myocarditis can be diagnosed by PCR as a rapid method [9–11]. Given the fact that in endomyocardial biopsies, the detection of cytomegaloviruses would be regarded as a pathological finding [10], this can be regarded as the cause of death in cases of SIDS. In our study, the myocardial tissues had been fixed in neutral phosphate-buffered formaldehyde (pH 7.4) or Notox (Earth Safe Industries) for a maximum of 48 h. This was crucial for the extraction of intact nucleic acids. The ability to isolate intact viral DNA and RNA from paraffin-embedded tissue permits the analysis of several cardiotropic viruses.

Although the pathomechanisms of virus-induced myocarditis are still a matter of great scientific interest, in clinical practice it is a widely recognized fact that cytomegaloviruses

Table 2
Details of PCR conditions used to amplify viral genes; CMV; cyc

Primer	Denaturation (°C)	Annealing (°C)	Extension (°C)	Number of cycles
CMV 1	94 (60 s)	64 (60 s)	72 (90 s)	30 (first round)
CMV 2				25 (second round)
CMV 3				
CMV 4				
CycF	94 (60 s)	58 (60 s)	72 (60 s)	35
CycR				

Fig. 1. Cytomegalovirus DNA detection in myocardial tissue from the interventricular septum (No. 9) of a 4-month-old boy, first regarded as sudden infant death syndrome (SIDS); 1+12=ladder; 2–8 and 10=samples from a cytomegalovirus-negative case; 11=+contr.

are possibly cardiopathogenic and can induce a myocarditis when detected in endomyocardial biopsy samples. In a recently published population-based study cytomegaloviruses were found to be the most common specific finding in immunocompetent patients with fatal myocarditis [12]. This is surprising because up to now cytomegalovirus-induced myocarditis was thought to be a finding, detectable only occasionally. Nevertheless these findings as well as our results emphasize the importance of the PCR-based diagnosis in cases of SIDS.

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