Meta-analysis of the association of enteroviruses with human heart disease

Christina Baboonian, Tom Treasure

Abstract
The role of viruses in the genesis of both dilated cardiomyopathy (DCM) and acute myocarditis remains uncertain. Modern molecular techniques such as polymerase chain reaction (PCR) and in situ hybridisation are sensitive means of detecting viral genomic material in human myocardial tissue and may help to resolve the quest. Meta-analysis of the papers in the literature records studies of both acute myocarditis and DCM where molecular techniques were used to demonstrate enteroviruses. This review studies information from the published literature as well as statistical analysis of the cumulative molecular data relating enteroviruses to DCM, and to compare these findings with the information available on the role of enteroviruses in acute myocarditis. Twelve papers reported studies in acute myocarditis, of which 11 found higher percentages of enteroviral RNA positivity in the diseased population, giving an overall odds ratio of 4.4. Seventeen papers reported studies in DCM, with 11 recording higher positivity rates in these patients. Cumulative analysis of these data suggests an overall odds ratio of 3.8. The causative role of enteroviruses in acute myocarditis, particularly in children, is supported by meta-analysis of the available literature. The data on DCM is suggestive of an association but a proportion of the studies are negative. (Heart 1997;78:539–543)

Keywords: enteroviruses; dilated cardiomyopathy; myocarditis; meta-analyses

Reports of an association between enteroviruses and myocarditis date back to 1956 when coxsackie B4 virus was isolated from the heart tissue of a neonate with fatal infection.1 Four decades later debate continues on the role of enteroviruses in acute and chronic myocardial disease.

Coxsackie B viruses are ubiquitous enteroviruses that spread rapidly within the community causing small epidemics with a high proportion of subclinical cases. Symptomatic infection ranges in severity from mild undifferentiated febrile illness or upper respiratory tract infection to severe systemic disease of neonates. Historically, it was the childhood cases of myocarditis that attracted attention to this group of agents.1–3 Early reports of epidemics in maternity homes where virus was isolated from peripheral sites of neonates,2,5 were followed by studies confirming the presence of coxsackieviruses in the heart tissue of infants with myocarditis.1,5 Later work showed that infection during pregnancy may result in fetal damage4–4 and large scale serological studies confirmed that mothers of infants with cardiological abnormalities were more likely to be infected with coxsackievirus B than were controls.3

Studies of a causal link between enteroviruses and adult cases of myocarditis proved more difficult. Virus isolation from adult heart tissue is very uncommon,9–11 and until recently many epidemiological studies relied on either virus culture from peripheral sites or serological investigations for diagnosis. As enteroviruses circulate freely in the community and virus can be cultured from the pharynx and faeces for several weeks following infection, diagnostic value cannot be attached to virus isolation from these sites. Serological studies are also fraught with difficulties as high titre antibodies can persist for prolonged periods in some individuals. Moreover, unlike young children, adults with prior exposure to enteroviruses may show a heterotypic rise in antibody titres confusing the diagnosis. Despite these problems, comparison of recent serological studies12–14 shows that 33–36% of adults with acute myocarditis have either rising titres of neutralising antibodies to coxsackie B viruses or have enterovirus specific IgM class antibodies indicative of a recent infection. The rate of infection in the control groups in these reports varies between 4% and 10%.

The advent of molecular techniques for the detection of viral RNA facilitated studies of heart tissue in non-fatal cases, providing an indication of the prevalence of these agents in acute myocarditis. Using in situ hybridisation, Hilton et al found 20% of childhood cases of acute myocarditis to carry entroviral RNA in heart tissue.15 This result was confirmed by Martin et al who, using polymerase chain
reaction (PCR), found eight of 38 heart biopsies taken from children with acute myocarditis and none of 17 controls carried entroviral sequences. Recent work by Towbin et al has extended these findings and shown that 23 of 135 paediatric patients were enterovirus positive by PCR.

The benefits of using molecular techniques were of particular importance in studies of adult cases of myocarditis. Some of the difficulties associated with the traditional methods of diagnosis were overcome with the widespread use of PCR. Although diverse rates of viral RNA detection in heart tissue have been reported, there is now general agreement that there is an association between coxsackievirus B infection and myocarditis. The techniques applied to the study of viral aetiology in acute disease have also been used in the study of dilated cardiomyopathy (DCM). It is the aim of this report to study information from the published literature and carry out statistical analysis of the cumulative molecular data relating enteroviruses to DCM and compare these findings with the information available on the role of enteroviruses in acute myocarditis.

### Methods

Analysis of the literature that records the use of molecular biological techniques such as PCR and in situ hybridisation for the detection of viral genomic material in acute myocarditis and DCM was carried out. Totally negative studies were included but those without a control group were excluded. The results were expressed as bar charts showing the percentage of samples found to be positive and the standard error of the binomial distribution.

### Results

In acute myocarditis, analysis of the molecular data for both adult and childhood cases shows that 11 of 12 studies reviewed record a higher percentage positivity in patients with DCM compared with controls (fig 1). The cumulative data from these studies shows that 23% of patients with disease (68 of 289 cases) and 6% of controls (14 of 216 individuals studied) were positive for entroviral sequences, giving an odds ratio of 4.4 (95% confidence interval (CI), 2.4 to 8.2).

Figure 2 summarises most recent studies of DCM where patients and control groups have been compared using molecular techniques. Overall, 406 patients with DCM and 438 control subjects have been investigated with approximately 23% of the patients and 7% of controls showing evidence of entroviral infection. The odds ratio was calculated to be 3.8 (95% CI, 2.1 to 4.6). The percentage positivity in patients with DCM varied between 0% and 75%, and 75%.

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### Figure 1
Evidence for association of enteroviruses with idiopathic myocarditis. The bar charts show the percentage of samples found to be positive with error bars showing the standard error of the binomial distribution. (X is the number of samples with enteroviral sequences and N the total number studied.)

### Figure 2
Evidence for association of enteroviruses with dilated cardiomyopathy. The bar charts show the percentage of samples found to be positive with error bars showing the standard error of the binomial distribution. (X is the number of samples with enteroviral sequences and N the total number studied.)
Discussion

Although an odds ratio of 3.8 is suggestive of an association between virus infection and DCM, on closer analysis, six of 17 reports found no significant differences between cases and controls (fig 2). Other studies included in this report used small numbers of patients making statistical analysis of the data difficult. Three of these investigations that found 20%, 28%, and 75% viral RNA carriage rates in the DCM group and no enteroviral sequences in the controls, included fewer than 10 patients. Five of the eight remaining reports that used PCR to detect viral material did not sequence the amplification products. As the amplification procedure is particularly prone to contamination, often from the reference strain used in the laboratory, sequencing of the products is informative, confirming the origin of the virus. In one study where sequencing was undertaken the amplification products were found to be identical to the coxsackievirus B3 positive control used in the routine assay. In another carefully controlled study where fivefold differences in enteroviral RNA positivity were recorded with sequencing data confirming the results, patients with inflammatory heart disease were grouped together with cases of DCM. Although this study has been included in the overall analysis of the role of enteroviruses in DCM, it is by no means certain that the positive patients were not cases of acute myocarditis.

A factor that further influences the interpretation of these studies is the choice of controls. As enteroviruses cause small epidemics there is likely to be variation in the virus carriage rates in different populations. In studies where serological analysis have been carried out often a significant difference in the IgM positivity rates between patients with DCM and controls have been observed. However, Keeling et al. found that if the serological status of patients was compared with their own family members or close associates then 37% of the disease population and 28% of the controls were IgM positive. Taking into account the numbers involved in each group a relative risk of 1.5 can be deduced. In the same study risk seemed much higher if patients were compared with an unselected control population (33% of the patients with DCM and 5% of the controls). When conducting serological investigations it is possible to obtain samples from close relatives or work associates exposed to the same environment and therefore likely to have experienced similar infections. Such comparisons are impossible when heart biopsy samples are analysed for viral RNA. Epidemiological data on unmatched groups, for a virus known to cause small localised outbreaks, is most active during a low grade infection in man and against which viral proteins is at present unknown. Which component of the immune response is most active during a low grade infection in man and against which viral proteins is at present unknown.

An alternative hypotheses for which there is some epidemiological and experimental evidence is the possibility that infection with enteroviruses may trigger an exaggerated immune response not only to the viral antigens but to the host tissue. Experimental work on mice with severe combined immunodeficiency has shown that transfer of peripheral blood leukocytes of patients with chronic myocarditis to these animals causes an impairment of left ventricular function suggesting a cell medi-
ated autoimmune response in the patient. Damage by autoantibodies has also been considered with a number of studies reporting higher frequencies of antibodies directed against cardiac myosin in patients with DCM. Whether these antibodies cause cellular damage is unknown.

The precise role of each viral strain in acute or chronic infection is also uncertain. Virus isolations from fatal cases of myocarditis have not attempted to distinguish between clinical isolates but also in different species or strains of animals.

Experimental work on mice has shown that some strains of coxsackievirus B3 are more virulent than others and that pathogenicity may differ significantly not only between clinical isolates but also in different species or strains of animals.


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*Heart* 1997 78: 539-543
doi: 10.1136/hrt.78.6.539

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