Clinical and Prognostic Significance of Detection of Enteroviral RNA in the Myocardium of Patients With Myocarditis or Dilated Cardiomyopathy

Howard J.F. Why, MB, MRCP; Brendan T. Meany, MB, MRCP; Peter J. Richardson, MD, FRCP; Eckhard G.J. Olsen, MD, FRCP; Neil E. Bowles, PhD; Louise Cunningham, PhD; Colette A. Freeke, BSc; Leonard C. Archard, PhD, MRCP

Background Enteroviral RNA sequences have been demonstrated in the myocardium of patients with myocarditis or dilated cardiomyopathy from presentation to end-stage disease. The prognosis of heart muscle disease has not previously been evaluated in relation to the detection of enterovirus in myocardial biopsy tissue.

Methods and Results We studied 123 consecutive patients with heart muscle disease prospectively. Multiple endomyocardial biopsy samples taken from all patients during diagnostic cardiac catheterization were classified histologically and were examined for enteroviral RNA by use of an enterovirus group-specific hybridization probe. Three enterovirus-negative patients with cardiac amyloidosis were excluded from subsequent analysis. Enteroviral RNA sequences were detectable in 41 (34%) of the remaining 120 patients (group A), while 79 (66%) had no virus detected (group B). The groups did not differ significantly in age, sex, symptomatic presentation, or hemodynamic characteristics; duration of symptoms was significantly shorter in group A (7.8 ± 9.6 versus 14.9 ± 19.0 months, P < .05). At follow-up (mean, 25 months; range, 11 to 50 months), patients from group A had an increased mortality compared with those in group B (25% versus 4%, respectively; P = .02). Mortality was also statistically greater in patients with symptomatic cardiac failure (P = .02), those with elevated left ventricular end-diastolic pressures (P = .03), and those in New York Heart Association functional classes III and IV (P = .05). Multivariate regression analysis, however, showed that only the presence of enterovirus RNA and symptomatic heart failure were of independent prognostic value.

Conclusions These data demonstrate that the detection of enterovirus RNA in the myocardium of patients with heart muscle disease at the time of initial investigation is associated with an adverse prognosis and that the presence of enterovirus RNA is an independent predictor of clinical outcome.

Key Words • myocarditis • cardiomyopathy • biopsy • viruses • prognosis

There has been much discussion about the role of viruses in the pathogenesis of myocarditis and dilated cardiomyopathy, although the latter entity is still by definition a condition of unknown etiology. The coxsackievirus B group has been previously implicated by serological studies; however, attempts to detect this virus in the myocardium by immunofluorescence or to culture it from myocardial tissue have been largely unsuccessful. The development of group-specific molecular hybridization probes, however, has allowed the identification of viral RNA sequences in myocardial tissue from patients with heart muscle disease. Another report demonstrated that enterovirus RNA was detectable in patients with the histological appearance of myocarditis (either active or healed) or dilated cardiomyopathy. Subsequent investigation revealed that enteroviral RNA can persist to end-stage disease. In none of these studies was there evidence of enterovirus in the myocardium in a significant proportion of control patients.

We prospectively evaluated the role of enterovirus infection in the prognosis of patients with heart muscle disease over a 4-year period. In addition to routine clinical and histopathological assessment, multiple endomyocardial biopsy samples from all patients with suspected myocardial disease were evaluated for enterovirus infection by use of an enterovirus group-specific cDNA hybridization probe.

Methods

Patients and Clinical Assessment

The patient population studied comprised 123 consecutive patients admitted to King's College Hospital between March 1985 and January 1989 for the investigation and treatment of heart muscle disease. A full history was obtained from all patients before investigation, and the following details were recorded: duration of symptoms before presentation, history of preceding viral illness, history of ethanol abuse (defined as >80 g alcohol daily for >5 years), history of chest pain or arrhythmias, and symptoms of cardiac failure. Routine ECGs, chest radiographs, echocardiograms, and Holter monitoring were performed on all patients. The presence of cardiomegaly on chest radiographs and any ECG abnormalities, including left bundle branch block and ventricular arrhythmias, were recorded.
### Table 1. Comparison Between the “Dallas Criteria”\(^\text{20}\) for the Histological Grading of Myocarditis and the Modified Histological Classification Used in This Study

<table>
<thead>
<tr>
<th>Study Biopsy</th>
<th>Dallas Criteria</th>
<th>First Biopsy</th>
<th>Subsequent Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myocarditis</td>
<td>Myocarditis with or without fibrosis</td>
<td>Ongoing myocarditis</td>
<td></td>
</tr>
<tr>
<td>Healing myocarditis</td>
<td>Borderline myocarditis</td>
<td>Resolving myocarditis</td>
<td></td>
</tr>
<tr>
<td>Healed myocarditis/dilated cardiomyopathy</td>
<td>No myocarditis</td>
<td>Resolved myocarditis</td>
<td></td>
</tr>
</tbody>
</table>

**Invasive Studies**

Cardiac catheterization, including coronary angiography, was performed by the Judkins technique in all patients. One hundred twenty patients had routine right and left heart catheterization; significant coronary artery disease and valvar heart disease were excluded as the cause of cardiac dysfunction. Three patients underwent right heart catheterization only, with noninvasive assessment of left ventricular function: 1 because of age (6 years) and 2 because of severe left ventricular failure. Left ventriculography was performed in the right anterior oblique 45° projection, allowing calculation of the ejection fraction by use of a digitization technique.\(^{14}\)

Endomyocardial biopsy from either the right (40 patients) or left (83 patients) ventricle was performed on all patients at the time of catheterization by the long-sheath method with either King's or Cordis myocardial bioproses.\(^{15,16}\) At least three biopsy samples (1 to 5 mg wet wt) were obtained per patient. Samples were divided for histological, histochemical, and enteroxival analysis and processed for analysis as previously described.\(^{17}\)

**Histological Analysis**

Histopathological examination of endomyocardial biopsy samples was carried out at the National Heart Hospital, London, by histochemistry and light and electron microscopy. Samples were classified on the basis of these findings into specific heart muscle disease or, according to strict criteria, into acute myocarditis, healing myocarditis, or appearances compatible with healed myocarditis or dilated cardiomyopathy.\(^{18}\) Briefly, acute myocarditis was diagnosed when the presence of acute inflammatory cells in close proximity to the myocardial fibers was associated with myocyte necrosis. In healing myocarditis, a chronic inflammatory cell infiltrate was still identifiable but not in direct contact with the myocytes; necrosis was absent, and increased interstitial collagen was present to a moderate degree. The changes of dilated cardiomyopathy were nonspecific but consisted of hypertrophic myocardial fibers, thickening of the endocardium, evidence of myocardial dilatation, and a variable degree of interstitial fibrous replacement.\(^{11,18,19}\) A comparison of this classification with that of the “Dallas criteria”\(^{20}\) is shown in Table 1.

**Analysis of Tissue for Enervoviral RNA**

Endomyocardial biopsy samples for viral examination were quick-frozen in liquid nitrogen and stored at -70°C until they were analyzed in batches. Detection of enterovirus RNA sequences was by molecular hybridization of total RNA extracted from myocardial tissue with a \(^{32}\)P-labeled enterovirus group-specific DNA probe in a quantitative slot-blot assay as described previously.\(^{11-13}\) This probe is complementary to nucleotide sequences in the 3' terminus of the coxsackievirus B2 genome and has previously been demonstrated to be highly specific for viruses of the enterovirus group.\(^{13}\) Samples were also hybridized with a control probe (cDNA clones complementary to the mRNA for \(\beta\)-tubulin for the first 17 patients, then to \(\beta\)6 for the remaining 106 patients\(^{21}\)) to quantify cellular RNA. The abundance of this latter messenger RNA (\(\beta\)6) is cell independent;\(^{21}\) it has been shown recently to encode the human ribosomal protein L41.\(^{22}\) The densitometric signals after autoradiography of blots hybridized with either the enterovirus probe or the control probe were expressed as a ratio (hybridization index) to control for differences between batches in (1) the concentration and specific activity of labeling of probes used in the hybridizations and (2) the autoradiography time for optimum densitometry. Similar analyses were performed concurrently on tissue from patients with myocardial disease of known origin (eg, coronary disease, specific heart muscle disease) as controls.

Approximately 30 endomyocardial biopsy samples and 15 control samples were analyzed in each batch. Biopsies were reported as positive for the presence of enteroviral RNA if the hybridization index exceeded the mean of the internal control group plus 3 SD (a statistical probability of a false-positive result of \(P<.002\)). At least three specimens were analyzed for enterovirus sequences from each patient. Because of the focal nature of the pathological findings, a patient was classified as enterovirus-positive if the hybridization index met the above criteria in any of their biopsy samples.

It was not considered ethical to do a biopsy of normal ventricles as controls during this study. Data to date, however, indicate that the detection of enterovirus RNA sequences in cardiac tissue from patients with diagnoses other than myocarditis or dilated cardiomyopathy is a rarity.\(^{23}\)

**Follow-up**

Patients were followed up in the routine outpatient clinic with frequency of review according to their clinical status. Details of subsequent deterioration, death, or cardiac transplantation were recorded. A small number of patients came from outside the United Kingdom, but, when possible, follow-up details were obtained from their referring physician. Repeat cardiac catheterization and endomyocardial biopsy were performed only when clinically indicated for follow-up of myocarditis or for symptomatic deterioration. In these patients, endomyocardial tissue was again examined for the presence of enteroviral RNA. The duration of follow-up from the time of cardiac catheterization was recorded to the nearest whole month for each patient.

**Statistical Analysis**

Comparison of clinical and hemodynamic variables between patients with and without detectable enterovirus RNA in endomyocardial biopsy tissue was performed with either \(\chi^2\) analysis or an unpaired \(t\) test as appropriate. Survival curves were calculated by the method of Kaplan and Meier.\(^{24}\) Statistical analyses of survival were performed on a desktop PC using the BMDP statistical package (BMDP Statistical Software, Inc). Univariate analyses of the effect of categorical variables on survival were performed with the Mantel-Cox test. Patients with missing data for any variable were excluded from the analysis of that variable. Variables were entered into a Cox proportional-hazards regression model in order of univariate significance to identify those variables that were predictive of outcome. Except where otherwise stated, death and cardiac transplantation are taken as the end points for survival analyses. All tests of significance are two-tailed. Values are quoted as mean±SD.

**Ethical Considerations**

The protocol for the analysis of human endomyocardial samples for enterovirus was reviewed and approved by the
ethical committee at King's College Hospital. All patients gave informed consent for all of the invasive studies performed.

Results

The mean age of the 123 patients studied was 44.9 years (range, 6 to 70 years); 94 patients were male and 29 female. Mean duration of symptoms before presentation was 14 months (range, 1 to 99 months), with the major symptoms on presentation being dyspnea (58%) and chest pain (20%). A history of preceding viral illness was obtained from only 16 patients, and 12 admitted to excessive alcohol consumption in the past. All were abstinent at the time of study and during the follow-up period. ECG evidence of cardiac arrhythmias was documented in 30 patients, and atrioventricular block was present in 5.

Histological examination of the endomyocardial biopsy samples showed that 3 patients had specific heart muscle disease (cardiac amyloidosis in all cases); they were excluded from further analysis. All were negative for the presence of enteroviral RNA. The histological diagnosis in the remaining 120 patients was acute myocarditis (7 patients), healing myocarditis (36 patients), and healed myocarditis/dilated cardiomyopathy (77 patients). These patients were divided into two groups on the basis of the presence or absence of enteroviral RNA sequences in endomyocardial biopsy specimens. Group A consisted of 41 patients (34%) in whom viral RNA was detectable in at least one biopsy specimen (EV positive), whereas no virus was detectable in any of the samples from the 79 patients (66%) in group B (EV negative). Results from a typical batch of 30 biopsy samples and 14 control samples showing the densitometric signals for the enterovirus probe, the 7B6 probe, and the respective hybridization indexes are given in Table 2. The results are displayed graphically in Fig 1, demonstrating the cutoff point between positive and negative results. A sample slot blot is shown in Fig 2.

The clinical variables of the two groups are shown in Table 3. The only significant difference was in duration of preceding symptoms, which was shorter in group A (EV positive) than group B (7.8±9.6 versus 14.9±19.0 months, t=1.98, P<.05). Table 4 details the hemodynamic variables for the two groups obtained at the time of cardiac catheterization and the breakdown of patients undergoing right or left ventricular biopsy. No statistical difference was found between the groups in terms of their hemodynamics, nor was there a difference in the proportion of positive tests between right and left ventricular biopsies. The histological classification of the patients in each group is shown in Table 5. There was no difference between right and left ventricular biopsies in the proportions in each histological category. Although there was a trend for patients in group A to have more active inflammation (5 of 41 with acute myocarditis versus 2 of 77 for group B), there was no statistically significant difference between the groups. A total of four patients were lost to follow-up after discharge from the hospital (1 from group A and 3 from group B). The mean duration of follow-up for the remainder was 25 months (range, 11 to 50 months). Mean length of follow-up did not differ between the two groups. Four patients underwent orthotopic cardiac transplantation during the follow-up period, 1 from group A and 3 from group B.

<table>
<thead>
<tr>
<th>Patient</th>
<th>7B6 Probe</th>
<th>Enterovirus Probe</th>
<th>Hybridization Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2292</td>
<td>640</td>
<td>0.28</td>
</tr>
<tr>
<td>2</td>
<td>920</td>
<td>480</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>632</td>
<td>264</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>1284</td>
<td>884</td>
<td>0.69</td>
</tr>
<tr>
<td>5</td>
<td>652</td>
<td>384</td>
<td>0.53</td>
</tr>
<tr>
<td>6</td>
<td>1368</td>
<td>600</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>1792</td>
<td>928</td>
<td>0.52</td>
</tr>
<tr>
<td>8</td>
<td>976</td>
<td>356</td>
<td>0.36</td>
</tr>
<tr>
<td>9</td>
<td>2200</td>
<td>932</td>
<td>0.42</td>
</tr>
<tr>
<td>10</td>
<td>480</td>
<td>204</td>
<td>0.42</td>
</tr>
<tr>
<td>11</td>
<td>1268</td>
<td>700</td>
<td>0.55</td>
</tr>
<tr>
<td>12</td>
<td>264</td>
<td>368</td>
<td>1.39</td>
</tr>
<tr>
<td>13</td>
<td>704</td>
<td>352</td>
<td>0.50</td>
</tr>
<tr>
<td>14</td>
<td>1588</td>
<td>868</td>
<td>0.55</td>
</tr>
<tr>
<td>15</td>
<td>524</td>
<td>216</td>
<td>0.41</td>
</tr>
<tr>
<td>16</td>
<td>884</td>
<td>92</td>
<td>0.10</td>
</tr>
<tr>
<td>17</td>
<td>334</td>
<td>412</td>
<td>1.23</td>
</tr>
<tr>
<td>18</td>
<td>324</td>
<td>504</td>
<td>1.55</td>
</tr>
<tr>
<td>19</td>
<td>800</td>
<td>604</td>
<td>0.75</td>
</tr>
<tr>
<td>20</td>
<td>412</td>
<td>480</td>
<td>1.16</td>
</tr>
<tr>
<td>21</td>
<td>472</td>
<td>356</td>
<td>0.75</td>
</tr>
<tr>
<td>22</td>
<td>1632</td>
<td>1472</td>
<td>0.90</td>
</tr>
<tr>
<td>23</td>
<td>628</td>
<td>736</td>
<td>1.17</td>
</tr>
<tr>
<td>24</td>
<td>532</td>
<td>1540</td>
<td>2.89</td>
</tr>
<tr>
<td>25</td>
<td>952</td>
<td>520</td>
<td>0.54</td>
</tr>
<tr>
<td>26</td>
<td>1568</td>
<td>1060</td>
<td>0.67</td>
</tr>
<tr>
<td>27</td>
<td>1324</td>
<td>724</td>
<td>0.55</td>
</tr>
<tr>
<td>28</td>
<td>2480</td>
<td>1328</td>
<td>0.54</td>
</tr>
<tr>
<td>29</td>
<td>2228</td>
<td>804</td>
<td>0.36</td>
</tr>
<tr>
<td>30</td>
<td>3440</td>
<td>1184</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Control subject

C1 1232 556 0.45  
C2 992 812 0.82  
C3 4308 860 0.20  
C4 3576 1220 0.30  
C5 9196 3336 0.36  
C6 6672 1456 0.21  
C7 280 196 0.70  
C8 572 192 0.33  
C9 1392 1008 0.72  
C10 1012 912 0.90  
C11 3156 1388 0.44  
C12 1436 504 0.35  
C13 1180 524 0.44  
C14 3428 1066 0.30  

Values are the densitometric readings for the enterovirus and 7B6 probes and the calculated hybridization indexes. These results are displayed graphically in Fig 1. Mean of controls, 0.44; SD of controls, 0.22. Positives classified as those with hybridization ratio >mean+3 SD of controls=0.44+0.66=1.1.
received immunosuppressive therapy. The medical management of both groups of patients was identical.

Repeat biopsies were performed in 16 patients (6 group A, 10 group B) at a mean interval of 11 months (range, 3 to 22 months) after the initial biopsy. In group A, virus was no longer detectable in 4 patients, the change being associated with clinical improvement in 3 of these. Both patients who remained positive subsequently died. No patient from group B was found to have enterovirus RNA on repeat biopsy.

Thirteen patients (11.2%) died during the follow-up period. All deaths were cardiac in nature. The mode of death was ventricular fibrillation in 4 patients (all group A), progressive cardiac failure in 3 (2 group A, 1 group B), and posttransplantation in 1 (group B); no precise diagnosis was recorded in the other 6 cases. A significantly greater proportion of deaths occurred in group A (n=10; 25% of the group) than in group B (n=3; 4% of the group) (Mantel-Cox test statistic, 5.42; P=.02). If cardiac transplantation is taken as an additional end point, then 16 of 116 patients (13.8%) progressed to this stage during follow-up, and the statistical difference between the two groups is preserved (11 of 40 reaching end stage from group A compared with 5 of 76 from group B; Mantel-Cox statistic, 5.40; P=.02). Survival curves for groups A and B are shown in Fig 3.

The significance of other parameters previously demonstrated to influence survival25-32 was assessed in a similar fashion, and the following results were obtained. Fifteen of 16 patients who died or progressed to transplantation were in New York Heart Association (NYHA) functional class III or IV. In all, end point was reached by 15 of 86 patients in NYHA class III or IV compared with only 1 of 30 in class I or II (Mantel-Cox statistic, 3.88; P=.05). Similarly, the presence of symptomatic cardiac failure at the time of cardiac catheterization (defined as the presence of two or more of the following: peripheral or sacral edema, elevated jugular venous pressure, a gallop rhythm, and bilateral basal crackles on chest auscultation) had a significant influence on survival on univariate analysis (Mantel-Cox, 5.71; P=.02), as did an elevated left ventricular end-diastolic pressure (Mantel-Cox, 4.45; P=.03). Mortality was higher in those patients with radiographic cardiomegaly (cardiothoracic ratio, >0.5) than in those with normal-size hearts, but the difference failed to reach statistical significance (Mantel-Cox, 1.60; P=.21). The same was true of ejection fraction, which appeared to have no significant effect on survival (Mantel-Cox, 1.94; P=.16). Neither the age of the patient (P=.16) nor
duration of preceding symptoms ($P = .36$) had any discernible effect on survival.

Pairwise Mantel-Cox analyses were performed with the data stratified according to the other variables that appeared to influence survival. When this was done for each factor, the presence of enterovirus appeared to retain its significant effect on prognosis (see Table 6). Multivariate stepwise regression analysis using the Cox proportional-hazards regression model identified only two variables, clinical heart failure and the presence of enterovirus RNA in the myocardium, as statistically significant independent predictors of outcome. The improvements in statistical significance obtained after the addition of each variable into the model are shown in Table 7. The addition of further variables failed to add significantly to the nonsurviving proportion.

**Discussion**

A number of clinical factors have been shown to be predictors of prognosis in patients with myocardial dis-
TABLE 5. Histological Classification of Patients in Groups A and B

<table>
<thead>
<tr>
<th></th>
<th>Group A (Enterovirus Positive) (n=41)</th>
<th>Group B (Enterovirus Negative) (n=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myocarditis</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Healing myocarditis</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>Healed myocarditis/dilated cardiomyopathy</td>
<td>25</td>
<td>52</td>
</tr>
</tbody>
</table>

χ²=4.63; P=NS.

ease. Most published studies concentrate on hemodynamic aspects, with reduced ejection fraction being viewed as the most important determinant of decreased survival.25-28 Since myocarditis appears to progress to dilated cardiomyopathy in only a proportion of cases, however, the prognostic value of poor left ventricular function may be dependent on the point in the natural history of the disease at which it is measured.29 Other factors that have been reported to indicate a poor prognosis include elevated intracardiac pressures,25,27,29,31,32 increased heart size,27,33 NYHA class III or IV,25,26,28,32 duration of preceding symptoms,32 and the presence of arrhythmias or conduction abnormalities.29 The majority of studies have failed to show that histology alone, however detailed, correlates with prognosis.25,29,34

Some serological evidence links acute myocarditis and positive serology for viruses of the coxsackievirus B group with a poorer prognosis,35 although in a 23-year follow-up study of 18 cases of coxsackievirus myopericarditis, no deaths were attributable to progressive cardiac failure in the absence of coronary artery disease.36 This apparent disparity might be partially explained by the lack of myocardial involvement during the initial presentation of these patients. Only 2 of 18 had signs and symptoms of cardiac failure, whereas 14 had a pericardial friction rub suggesting acute pericarditis. Only 2 of the surviving patients had abnormal left ventricular ejection fraction responses to exercise, a much lower proportion than that of Quigley et al30 in their follow-up study of acute myocarditis. In addition, we previously demonstrated no correlation between positive serum μ-antibody capture ELISA for coxsackievirus B1 through B5 IgM and the presence or absence of enteroviral RNA sequences in the myocardium of patients with myocarditis or dilated cardiomyopathy.37

The association between alcohol abuse and heart muscle disease is well established.38,39 Although a causative relation has been harder to establish, evidence from myocardial enzyme studies points to a specific alcoholic heart muscle disease.40 Complete resolution of symptoms with return to normal of cardiac size and function may occur with subsequent abstinence.39 We have demonstrated that enterovirus RNA sequences may be found in the myocardium of some alcohol abusers with dilated cardiomyopathy; this may explain the failure of some patients to improve despite complete abstinence from alcohol intake.

The overall survival in the present study was approximately 80% at 2 years. This is consistent with the results of both the Veterans Administration41 and the Framingham42 studies of patients with all grades of chronic heart failure. We have further demonstrated that the detection of enterovirus RNA in myocardial tissue of patients with either myocarditis or dilated cardiomyopathy is of prognostic value. This applies not only when death alone is considered as the end point during follow-up but also if progression to cardiac transplantation is included. The presence of virus appears to be as strong a predictor of poor prognosis as NYHA functional class, elevated intracardiac pressures, or the presence of clinical heart failure. Moreover, multivariate analysis reveals the presence of enteroviral RNA in the myocardium at the time of initial biopsy to be of independent prognostic significance.

The difference in duration of symptoms before presentation between groups A and B is of considerable interest. It is likely that, on average, patients of both groups were investigated at approximately the same point in the clinical course of their illness. The shorter length of illness in the patients from group A may therefore have two implications. First, it appears that patients of group A (in whom enterovirus RNA was detected) have a more rapid clinical decline than those in group B, suggesting that the presence of enterovirus RNA disposes to a more aggressive illness. Second, if survival analysis were calculated from the time of initial illness (at the time of presumptive infection), then the difference in prognosis between the groups would be even greater, assuming that the mortality rates of the groups do not change. Alternatively, viral heart disease may follow a biphasic pattern: the initial phase would be characterized by the presence of virus within the myocardium and a high mortality (equivalent to group A), with a second phase occurring after a variable interval when virus is no longer detectable in myocardial tissue and clinical decline is less marked (equivalent to group B). Although this hypothesis is attractive, particularly in view of our limited data on repeat biopsy, it is not without drawbacks, especially the assumption that all cases of heart muscle disease have a viral trigger at some

![Proportion surviving](https://example.com/proportion.png)

**Fig 3.** Survival curves for enterovirus-positive (solid line) and enterovirus-negative (dashed line) groups, with proportions calculated according to the method of Kaplan and Meier.
point. Even if true, this hypothesis does not reduce the prognostic significance of virus detection and may even enhance it.

Our failure to demonstrate any prognostic significance by univariate or multivariate analysis for reduced ejection fraction contrasts with previous studies, although in fact mortality was higher in the group of patients with reduced ejection fractions. Several factors may account for this difference. The cutoff for diminished ejection fraction was taken at ≤40%, in line with that considered as showing considerable impairment in other studies and representing a value approximately 3 SD below the normal population mean. It appears, however, that only severe left ventricular impairment with an ejection fraction <25% to 30% may be of predictive value; in the latter study, multivariate analysis demonstrated no independent prognostic value for ejection fraction in those patients whose ejection fractions were <30%. Second, in a mixed population of patients with myocarditis and dilated cardiomyopathy, ejection fraction at the time of invasive investigation is less likely to be of value in predicting outcome, since those patients with myocarditis may well undergo spontaneous improvement of left ventricular function over the succeeding months. Indeed, Quigley et al. suggested that, in patients with acute myocarditis, long-term outcome was predicted by assessment of left ventricular function 6 to 8 months after the initial presentation. Finally, up to 50% of deaths from dilated cardiomyopathy are due to sudden death (4 of 8 in this study for whom the mode of death was documented) rather than progressive cardiac failure. The incidence of sudden death does not appear to correlate with the cardiac hemodynamics in these patients.

It appears to be unimportant, in regard to enterovirus detection, whether endomyocardial biopsy samples are taken from the right or left ventricle. In the present study, the rates of enterovirus RNA detection were almost identical, with 13 positives in 40 patients undergoing right ventricular biopsy (33%) compared with 28 of 80 patients (35%) in whom left ventricular samples were taken. The percentage of patients in each histological group was not influenced by the ventricle biopsied. In this study, repeat biopsy was performed in only a small number of patients. When virus could no longer be detected in biopsy tissue, some clinical improvement in heart failure was observed; this may reflect clearance of the virus from the myocardium. The small number of patients who had repeat biopsies, however, precludes statistical analysis of these findings.

The expression of 7B6 is depressed in samples that concomitantly give a strong signal with the enterovirus-specific probe (Table 2, Fig 2). This is similar to the findings of Lohr and Oldstone, who showed that persisting cytomegalovirus infection of the pancreas in patients with type 2 diabetes mellitus downregulated the expression of 28S ribosomal RNA. It is interesting that in the present study also, the control probe was directed against a structural component of the ribosome. It is reasonable to speculate that enterovirus persistence within the myocardium causes modulation of the expression of the gene for 7B6, although the absolute hybridization signal with the enterovirus probe is increased.

Several studies that used the polymerase chain reaction (PCR) to detect enterovirus RNA in endomyocardial biopsy samples support our observations, although the frequency of detection was variable. We have subsequently used primers specific for the conserved enterovirus 5' nontranslated region to amplify, by nested PCR, sequences from a small number of analogous tissue samples. When positive by PCR, direct nucleotide sequencing showed that the amplified product has greatest homology to the 5' nontranslated region of coxsackievirus B (unpublished data).

The precise mechanism by which enterovirus infection affects prognosis, whether by altering myocardial function or by inducing arrhythmias, remains a matter for speculation. Recent preliminary evidence, however, indicates that enterovirus may persist in a defective, mutant form, which may account for the lack of inflammatory response seen in dilated cardiomyopathy. This defective virus may affect prognosis by modulating cardiac gene expression, a process that has been shown to occur in the failing heart. Additionally, virus persistence may initiate a secondary, immune-mediated process that results in a deterioration in cardiac function.

Acknowledgment

The authors gratefully acknowledge the help of Fiona Reid for her statistical advice.

References


Clinical and prognostic significance of detection of enteroviral RNA in the myocardium of patients with myocarditis or dilated cardiomyopathy.
H J Why, B T Meany, P J Richardson, E G Olsen, N E Bowles, L Cunningham, C A Freeke and L C Archard

Circulation. 1994;89:2582-2589
doi: 10.1161/01.CIR.89.6.2582

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/6/2582

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/