Myocardial Enterovirus Infection With Left Ventricular Dysfunction: A Benign Disease Compared With Idiopathic Dilated Cardiomyopathy

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Objectives. Endomyocardial biopsy samples from patients with idiopathic dilated cardiomyopathy were screened for the presence of enterovirus genome. Patients with enterovirus-positive samples were further studied with regard to disease course, histologic variables and response to interferon-alpha treatment.

Background. Studies of patients with idiopathic dilated cardiomyopathy have reported widely divergent clinical outcomes, suggesting that there is no unique underlying pathogenetic mechanism.

Methods. Five left ventricular endomyocardial biopsy samples were screened for the presence of the enterovirus genome by an established in situ hybridization technique in combination with a histologic, histomorphometric and immunohistologic workup. The course of the disease was then prospectively followed for up to 50 months. Virus-positive patients whose condition deteriorated were treated with interferon-alpha.

Results. Of 77 patients, 20 (26%) had enterovirus-positive and 57 (74%) enterovirus-negative biopsy samples. During a mean follow-up period of 25.8 ± 13.7 months, 1 patient in the enterovirus-positive group and 11 in the enterovirus-negative group died. Four patients in the enterovirus-negative group underwent heart transplantation (p < 0.05). The surviving 19 enterovirus-positive patients had a decrease in mean left ventricular end-diastolic diameter from 66 to 61 mm (p < 0.05) and a mean increase in left ventricular ejection fraction from 0.35 to 0.43 (p < 0.05). In contrast, enterovirus-negative patients had no significant change in end-diastolic diameter or left ventricular ejection fraction. Four patients in the enterovirus-positive group whose condition deteriorated were treated with a 6-month course of subcutaneous interferon-alpha (3 × 10⁶ U every second day). This treatment induced hemodynamic improvement in all four patients and eliminated the persistent enteroviral infection in two.

Conclusions. Enterovirus-positive patients have a better heart transplantation-free survival rate and hemodynamic course, with fewer histologic changes, than do enterovirus-negative patients. In addition, enterovirus-positive patients respond favorably to interferon-alpha treatment. These observations indicate that myocardial enteroviral infection with associated left ventricular dysfunction is a distinct disease entity with a benign course.

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Idiopathic dilated cardiomyopathy is diagnosed by the exclusion of any known disease that might induce heart muscle dysfunction (1). The hemodynamic course of idiopathic dilated cardiomyopathy is variable; spontaneous hemodynamic improvement and stabilization can be expected in ~34% to 39% of all patients (2,3). Therefore, it is assumed that the pathogenesis of the disease is not unique and represents the end stage of various cardiopathogenic factors, such as genetic disorders, nutritional defects, toxins, drugs and viral infections (4). Virus isolation in endomyocardial biopsy samples from patients with idiopathic dilated cardiomyopathy has been previously unsuccessful (5). However, recent developments in modern molecular biologic techniques, such as in situ hybridization and polymerase chain reaction, can detect enteroviral

genomes in patients with idiopathic dilated cardiomyopathy and myocarditis (6-14). Although in situ hybridization techniques have a very high sensitivity for enteroviral detection—as few as 20 viral copies are detectable (8)—sampling errors during endomyocardial biopsy may lead to underestimation of the percent of viral infection in patients with idiopathic dilated cardiomyopathy. In murine models of viral myocarditis, persistent infection of ~0.01% of myocardial cells (15) could be shown, whereas the inflammatory reaction had largely subsided in the chronic stage of the disease (16). Thus, it is becoming evident that a high proportion of human idiopathic dilated cardiomyopathy might develop from acute enteroviral myocarditis (17-19).

Because the inflammatory reaction seems to be related more to active virus replication than to autoimmunity (15), immunosuppressive therapy in idiopathic dilated cardiomyopathy has become irrational; this is supported by the proved ineffectiveness of immunosuppressive therapy in placebo-controlled trials of myocarditis (20) and idiopathic dilated cardiomyopathy (21). However, coxsackievirus B3 replication can be inhibited by interferon in fetal human heart cells (22), human myocardial fibroblast cultures (23) and murine models

of encephalomyocarditis (EMC) virus and coxsackievirus B3 myocarditis (24–26). The present study aimed to described the hemodynamic and clinical course of enterovirus-positive versus enterovirus-negative patients with idiopathic dilated cardiomyopathy. In addition, myocardial histomorphometric and immunohistologic variables, as related to the virus infection, were investigated as well as the effect of interferon-alpha therapy in those enterovirus-positive patients with hemodynamic deterioration.

Methods

Study protocol. Between July 1987 and May 1991, 80 patients with left ventricular dysfunction of unknown origin according to World Health Organization/International Society and Federation of Cardiology (WHO/ISFC) definitions (1) were included in a longitudinal prospective trial. The observation period was completed in September 1992. The investigators had knowledge of the results of enterovirus detection.

The following diagnostic procedures were performed on entry into the study: left heart catheterization; left ventricular endomyocardial biopsy; echocardiography; gated blood pool scanning; Swan-Ganz catheterization; standard laboratory tests; and a clinical and comprehensive anamnestic workup with determination of duration of preclinical symptoms, such as the patient's subjective impairment (e.g., dyspnea, angina pectoris, palpitations) or objective variables (e.g., first documentation of a left bundle branch block, pathologic echocardiographic findings). Because of the large number of enteroviruses (~70 distinct serotypes) that have been associated with human viral heart disease and the unspecificity of the results, serologic antibody studies for enteroviral detection were not performed.

Follow-up examinations were performed in subsequent 12- to 18-month intervals and included Swan-Ganz catheterization at rest and during exercise, gated blood pool scanning, clinical and anamnestic workup, standard laboratory tests, echocardiography and electrocardiography.

Techniques. Left heart catheterization (Judkins technique), Swan-Ganz catheterization and gated blood pool scanning were performed according to standard techniques (2).

Cardiac biopsy. Biopsy samples were obtained from the posterolateral free wall of the left ventricle with a Cordis long sheet bioptome (5.4F, 7F). At least five biopsy samples were obtained and fixed by immersion (four samples with 4% buffered formalin, one sample in 1.5% glutaraldehyde and 1.5% formaldehyde buffered with 0.2 mol phosphate buffer). The formalin-fixed biopsy samples were used for immunohistochemistry and in situ hybridization techniques as follows: Immunohistochemical analysis was performed on paraffin sections to characterize inflammatory infiltrates. In each case, L-CA (CD 45; panleucocyte; Dakopatts, Copenhagen, Denmark), KP 1 (CD 68; macrophages, monocytes, myeloid cells; Dakopatts), L 26 (B-cells, Dakopatts) and MT 1 (T cells; Biotest AG, Dreieich Germany) were used as primary antibodies. Binding was detected with the alcalic phosphatase–

antialcalic phosphatase (APAAP) method. The number of positive cells/mm^2 of sectional area was determined. Morphometric investigations were performed on paraffin sections. Hematoxylin-eosin–stained sections were used for measurements of the fiber diameters. The diameters were measured on cross sections of myofibers in the nuclear region. In the case of eccentric section profiles, the short diameter was chosen. Forty profiles/biopsy were evaluated. The volume fractions of interstitial space and collagen were determined according to standard morphometric techniques with point counting on sections stained with Sirius red (special collagen stain).

In addition, the sample fixed with glutaraldehyde and formaldehyde was embedded in plastic (Epon-Araldite), and 0.5-μm semithin sections were stained with alcaline toluidine blue and counterstained with paraphenylene diamine. The intracellular volume fraction of myofibrils was determined by point counting, as described elsewhere (2). The classification of myocarditis was performed on the basis of the "Dallas" criteria (27).

In situ hybridization of the endomyocardial biopsy samples was performed according to techniques previously described in detail by Klingel et al. (15) and Kandolf et al. (28). Briefly, serial sections (4 μm) were mounted on microscope slides, dewaxed from paraffin and hybridized with a sulfur-35–labeled coxsackievirus B3 DNA probe (200 μg/ml). The probe contained a virus-specific 6.2-kilobase (kb) and a 1.0-kb fragment corresponding to nucleotides 66 to 7128 of the full-length transcript of the coxsackievirus B3 viral genome. After hybridization and autoradiography, at least 6 to 10 slides with three to four formaldehyde-fixed biopsy samples were evaluated for the presence of enteroviral genomes.

Usually in patients several cells were tested positive. In the case of a single-cell infection, only those patients were classified as enterovirus positive, if this cell was positive in a consecutive tissue slice.

Study patients. *Patient selection.* Eighty patients were prospectively included in this study between July 1987 and May 1991. Each patient presented with either heart failure, arrhythmia or left bundle branch block of unknown origin. A precise workup of the patients could not identify an illness other than heart disease. Left heart catherization and coronary angiography demonstrated the presence of idiopathic dilated cardiomyopathy according to the definition of WHO/ISFC (1). Patients with coronary or valvular heart disease (except for mild mitral regurgitation), systemic hypertension, hypertrophic cardiomyopathy, predominant pericardial disease or systemic disease of the heart were not considered to have idiopathic dilated cardiomyopathy and were therefore excluded. For inclusion in the study, left ventricular ejection fraction <0.50 was required. All 80 patients were white (mean age 48.2 years, range 19 to 67; 64 men [80%], 16 women [20%]).

Patient classification. Patients were classified as having deteriorating idiopathic dilated cardiomyopathy if during the period between two examinations left ventricular ejection fraction decreased >0.05 or maximal mean pulmonary artery pressure increased >5 mm Hg, or both. Idiopathic dilated

Table 1. Clinical Characteristics of 77 Patients With Idiopathic Dilated Cardiomyopathy

	Enterovirus Positive (n = 20)	Enterovirus Negative (n = 57)	p Value
Age (yr)	47.2	48.6	0.63
% male	80	75.4	0.93
% incidence of left bundle branch block	30	39	0.68
Echo end-diastolic diameter (mm)	66	64	0.44
Alcohol consumption (%)			
None	40	31	
Rare	20	42	0.26
<50 g/day	30	15	
≥50 g/day	10	12	
Atrial fibrillation (%)	35	14	0.04
Treatment with ACEI (%)	80	81	0.74
NYHA functional class (%)			
I	20	16	
II	45	47	0.41
III	30	35	
IV	5	2	
Duration of preclinical symptoms (%)			
≤3 mo	33	29	
4–6 mo	14	14	0.91
>6 mo	53	57	
Mean (mo)	25	25	
LVEF at rest	0.35	0.34	0.85
Mean pulmonary artery pressure			
At rest (mm Hg)	21	21	0.96
At maximal work load (mm Hg)	39	44	0.22
Systolic arterial pressure (mm Hg)	131	130	0.57
Heart rate at rest (beats/min)	79	82	0.32

Data presented are mean values. ACEI = angiotensin-converting enzyme inhibitor; Echo = echocardiographic; NYHA = New York Heart Association.

cardiomyopathy symptoms were considered lessened if left ventricular ejection fraction increased by >0.05 or maximal mean pulmonary artery pressure decreased by 5 mm Hg, or both. Changes in left ventricular ejection fraction and pulmonary artery pressure within these limits were considered insignificant and did not result in a change in the patient's classification.

Alcohol intake was graded on a scale of 0 to 3 (0 = no alcohol intake; 1 = alcohol on rare occasions; 2 = <50 g alcohol/day, 3 = ≥50 g/day). No other drugs were consumed by any patient.

Severity of heart failure was classified according to New York Heart Association functional class.

Therapy. Patients were treated according to degree of heart failure with angiotensin-converting enzyme inhibitors, digitalis or diuretic agents. The treatment was comparable in both patient groups at the time of diagnosis (Table 1). Patients with previous tachycardiac syncope or resuscitation because of ventricular fibrillation received an automatic implantable cardioverter-defibrillator. Dicumarole was given as an oral anticoagulant agent in patients with left ventricular ejection fraction <0.35 or atrial fibrillation. Four patients with entero-virus-positive biopsy samples and hemodynamic deterioration

6 months after diagnosis received interferon-alpha$_{2a}$ treatment (Roferon A, purchased by Roche, Basel, Switzerland) in evening subcutaneous dosages, 3.0×10^6 U every other day.

The reinvestigation procedures and interferon treatment trial were approved by the University Hospital Committee of Ethics. Written informed consent was obtained from all patients for all left heart catheterization and biopsy procedures and interferon treatment.

Statistics. Results are expressed as mean value ± SD. Comparisons between the enterovirus-positive and enterovirus-negative groups were made by the unpaired Student t test after consideration of equal group variances by means of the F test. A p value < 0.05 was considered significant. All tests performed were two sided. The hemodynamic course in different groups was dichotomized as improved or unchanged/deteriorating and compared by the Fisher exact test. Other dichotomized variables were tested by the chi-square test. The variation of continuous variables within a group over time were tested by the paired Student t test. Survival rates were determined by the Kaplan-Meier method. Heart transplantation and death were censored. For differences between groups the log-rank test was used.

Results

Hemodynamic course and survival. Light microscopic evaluation of left ventricular endomyocardial biopsy samples from the 80 study patients revealed 3 (4%) with myocarditis (1 virus positive, 2 negative, all improved) and 77 who were diagnosed as having idiopathic dilated cardiomyopathy. Twenty patients (26%) were shown to be enterovirus positive and 57 (74%) negative by means of in situ hybridization. Although the clinical characteristics at the time of diagnosis revealed no differences between the two groups with regard to duration of preclinical symptoms or history of acute infective illness (except for atrial fibrillation, which was more frequent in the enterovirus-positive group) (Table 1), the course of the disease differed in both groups.

In the enterovirus-negative group, 11 patients (19%) died, and 4 (7%) underwent heart transplantation within a mean [±SD] follow-up period of 24.3 ± 13.0 months (6 patients died suddenly). In the enterovirus-positive group, one patient (5%) died of congestive heart failure within a mean follow-up period of 29.7 ± 14.4 months. No heart transplantation was necessary in this group. Thus, transplantation-free survival was significantly greater in the enterovirus-positive group (p < 0.05) (Fig. 1). During a mean reevaluation period of 16.2 ± 9.2 months in the enterovirus-negative group, hemodynamic function improved in only 10 patients (18%), was unchanged in 23 (40%) and deteriorated in 6 (11%); 3 patients did not appear for reevaluation but were still alive. During a mean reevaluation period of 19.2 ± 8.9 months in the enterovirus-positive group, hemodynamic function improved in 11 patients (55%), remained unchanged in 3 (15%) and deteriorated in 4 (20%) (p < 0.02), who later underwent interferon treatment (Fig. 2); 1 patient refused hemodynamic reevaluation but was still alive.
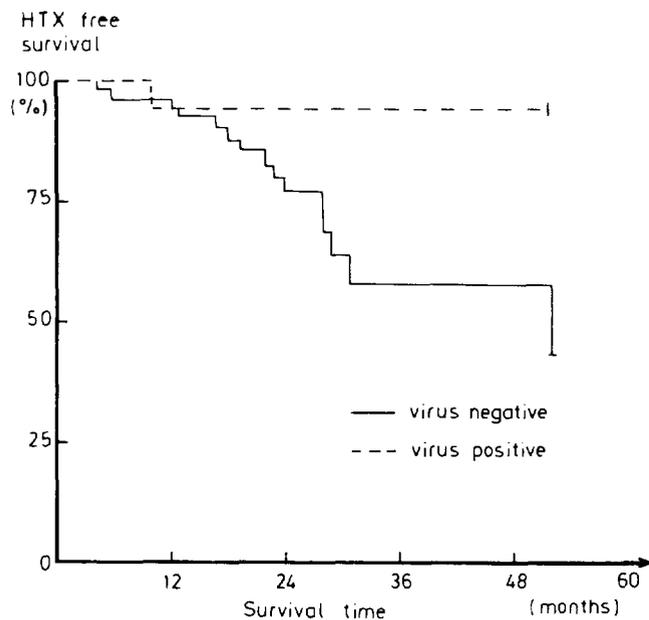
Figure 1. Heart transplantation (HTX)-free survival without interferon-alpha treatment in enterovirus-positive and enterovirus-negative patients with idiopathic dilated cardiomyopathy. Heart transplantation-free survival rate is higher in enterovirus-positive patients.

Left ventricular ejection fraction at rest increased significantly from 0.35 ± 0.13 to 0.43 ± 0.09 in the virus-positive group (p < 0.05) (Fig. 3) but was unchanged in the virus-negative group (0.34 ± 0.12 to 0.37 ± 0.14, p = NS). Maximal pulmonary artery pressure remained unchanged in both the virus-positive (39 ± 10 mm Hg [5.2 ± 1.3 kPa] vs. 36 ± 12 mm Hg [4.8 ± 1.6 kPa], p = NS) and virus-negative groups (44 ± 13 mm Hg [5.9 ± 1.7 kPa] vs. 45 ± 15 mm Hg [6.0 + 2.0 kPa], p = NS). However, according to echocardiographic end-diastolic diameter, virus-positive hearts became signifi-

cantly smaller (from 66 to 61 mm, p < 0.05) than virus-negative hearts, which remained unchanged (64 vs. 65 mm, p = NS).

Thus, according to the presence or absence of enterovirus within the myocardium, there was a striking difference in both hemodynamic course and major cardiac event rate (i.e., death or heart transplantation).

Endomyocardial biopsy. All endomyocardial biopsy samples were subjected to histomorphometric evaluation, and immunohistochemistry was used in a subset of samples (n = 30). The results of these tests are summarized in Table 2. The myofibril volume fraction (i.e., myofibril volume within a myocyte) was reduced in the enterovirus-negative group, but other histomorphometric features (myocyte diameter, interstitial volume fraction) did not differ. Immunohistologic studies revealed no striking differences; however, there was a trend to more macrophages and more panleukocytes, particularly T lymphocytes in the enterovirus-positive biopsy samples (Table 2).

Interferon-alpha treatment in a subset of enterovirus-positive patients. Endomyocardial biopsy samples from 20 patients were enterovirus positive according to in situ hybridization. Within 29.7 ± 14.4 months, 1 patient died, 1 was lost to follow-up, and 14 were in hemodynamically or clinically stable or improved condition within 19.2 ± 8.9 months. Therefore, none of these patients underwent interferon-alpha treatment, but four of them had hemodynamic deterioration in the mean 22.5 ± 5.0 months after diagnosis. They were treated with 3.0 × 10⁶ U of interferon-alpha (Roferon A) every other day subcutaneously for 6 months. Subsequent hemodynamic evaluation showed an increase in left ventricular ejection fraction in three patients; in one patient left ventricular ejection fraction was unchanged, but mean maximal pulmonary artery pressure was significantly reduced (from 43 to 31 mm Hg) at comparable ergometric work loads. Clinical status also improved by one functional class in all patients. No adverse reactions, except for a slight fever at the start of the

Figure 2. Flow diagram of the 80 study patients (pts) classified by in situ hybridization of left ventricular endomyocardial biopsy samples as having myocarditis or idiopathic dilated cardiomyopathy (DCM). The 57 enterovirus-negative (neg.) patients had a worse outcome during the mean observation period of 29.7 months than the 20 enterovirus-positive (pos.) patients. HTX = heart transplantation.
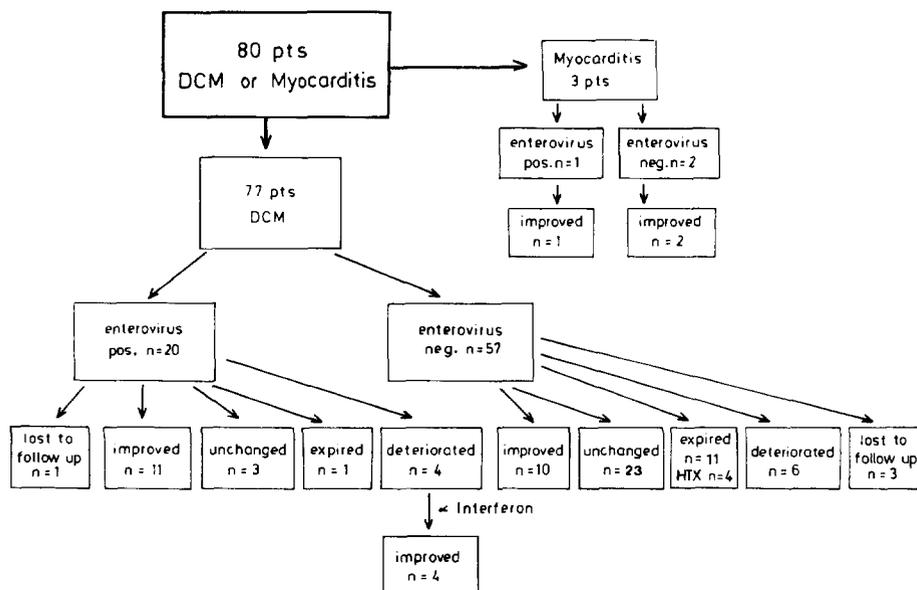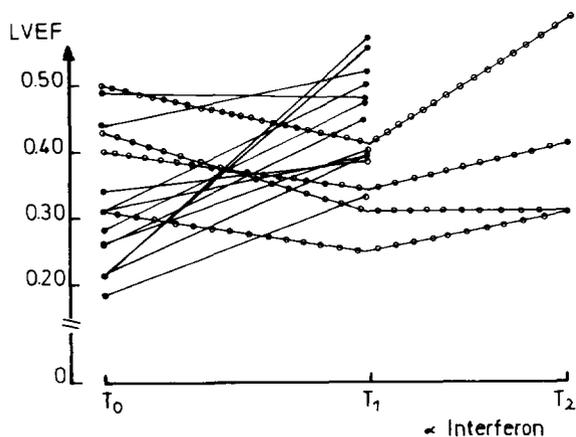
Figure 3. Changes in left ventricular ejection fraction (LVEF) for each enterovirus-positive patient with idiopathic dilated cardiomyopathy. The four patients with hemodynamic deterioration received subsequent treatment with interferon-alpha (3 × 10⁶ U subcutaneously every other day) for 6 months. Left ventricular ejection fraction increased in three patients and was unchanged in one but improved by Swan-Ganz catheter measurements during treatment (T) with interferon-alpha. **Solid lines with circles** = those patients whose condition deteriorated and who received interferon-alpha.

Table 2. Histomorphometric and Immunhistologic Endomyocardial Biopsy Results

	Enterovirus Postive		Enterovirus Negative		
	No.	Mean ±SD	No.	Mean ±SD	p Value
Myofibrillar volume fraction (%)	18	57.5 ± 4.3	53	55.1 ± 3.1	0.036
Interstitial volume fraction (%)	15	25.5 ± 11.6	45	24.8 ± 8.1	0.82
Myocyte diameter (μm)	17	27.7 ± 5.6	51	29.1 ± 5.1	0.37
Pan-leukocytes (1/mm²)	10	3.8 ± 3.5	20	2.4 ± 2.5	0.12
Macrophages (1/mm²)	10	2.7 ± 3.9	20	1.0 ± 0.70	0.24
T cells (1/mm²)	10	5.1 ± 5.3	20	3.4 ± 3.3	0.38
B cells (1/mm²)	10	0.2 ± 0.3	20	0.4 ± 0.20	0.52

treatment, were observed. Changes in left ventricular ejection fraction for all enterovirus-positive patients is shown in Figure 3 (n = 15). After completion of interferon treatment, subsequent left ventricular endomyocardial biopsy revealed the persistence of the enterovirus genome in two patients, even though they both had improved hemodynamic function.

Discussion

Course of idiopathic dilated cardiomyopathy as related to myocardial enterovirus infection. To our knowledge, the present study included the largest group of patients with idiopathic dilated cardiomyopathy to be screened by in situ hybridization techniques for enterovirus-specific ribonucleic acid (RNA) in left ventricular myocardium. Enterovirus RNA was present in 26% of patients by in situ hybridization, which is capable of detecting as few as 20 virus copies (8) and is considered a powerful tool with high sensitivity and specificity for studying viral infection. However, differing results have been obtained with various molecular biologic techniques, ranging from 0% to 52% of RNA detection in various patient populations and biopsy sites (6–14). Because the technique used in the present study has a high specificity, it is unlikely that the percent of RNA detection was overestimated; rather, it was probably underestimated because of the sampling error inherent in the endomyocardial biopsy technique (29).

Pathogenesis. The pathogenesis of virus-negative idiopathic dilated cardiomyopathy is unknown. Structural disintegrity is more pronounced than in the enterovirus-positive disease, as indicated by our measurements of the myofibril volume fraction in the endomyocardial biopsy samples (Table 2). As shown in the present study, virus-negative idiopathic

dilated cardiomyopathy is correlated with deteriorating left ventricular function and, consequently, an adverse cardiac event rate. No clinical or hemodynamic feature indicated a higher prevalence for enteroviral heart disease, except for atrial fibrillation, which occurred more frequently in enterovirus-positive patients (Table 1). Thus, the present study did not permit investigation into the mechanism that triggers or maintains the persistent enterovirus infection or identification of features that are associated with a higher likelihood of enterovirus left ventricular dysfunction (other than atrial fibrillation).

In a previous longitudinal study in 1985 (2), we found that "there are two groups of patients with idiopathic dilated cardiomyopathy that differ with respect to their hemodynamic course and morphologic features. This makes it more likely that the pathogenesis of the disease is not unique, and if it is not, the entity idiopathic dilated cardiomyopathy can be divided into a progressive and nonprogressive form." In the present study, we found that the virus-positive patients resembled those patients previously described with nonprogressive idiopathic dilated cardiomyopathy with a preserved myofibril volume fraction on endomyocardial biopsy. With the high prevalence of enterovirus infection in our subset of patients with idiopathic dilated cardiomyopathy, it is very likely that the morphologic and hemodynamic findings in the previous group were caused by the enterovirus infection and that myocardial enterovirus infection with left ventricular dysfunction is a distinct disease entity.

The partial reversibility or stabilization of the virus-induced left ventricular dysfunction might be explained by the spontaneous or therapeutic (interferon-alpha) elimination of the virus and the concomitant inflammatory reaction.

Interferon treatment in enterovirus-positive patients. Coxsackie virus B3 replication can be inhibited in cell cultures and murine myocarditis experiments (22–26). To our knowledge, the present study is the first to investigate the effect of interferon-alpha treatment in a subset of patients with enterovirus-induced progressive left ventricular dysfunction. The dosage applied, 3.0 × 10⁶ IU subcutaneously every other day for 6 months, was self-administered by the patients and

resembles concentrations that eliminated virus replication in human fibroblast cultures in a previous study of ours (30). The dosage is equivalent to that used in virus hepatitis B and C trials (31,32) and was well tolerated. There were only mild side effects: Three patients developed fever after the first injections, two complained of an influenza-like syndrome at the beginning, and one showed a transient elevation of transaminase levels. Although there are some case reports in which a higher dosage ($\sim$3 to 20 $\times$ 10^6 U/day) of interferon is suspected of causing reversible heart dilation (33,34) in patients without cardiac disease, on average in the present study, ejection fraction increased from 0.33 to 0.41, and mean pulmonary artery pressure at maximal work load decreased from 45 to 33 mm Hg. After a 6-month treatment course, two of four patients were still enterovirus positive by in situ hybridization on subsequent biopsy, even though progressive left ventricular dysfunction was reversed. Whether this was due to a reduction rather than elimination of the virus or to a concomitant macrophage reaction cannot be determined and warrants more investigation in larger groups of treated patients. However, the benign response to the interferon treatment in the present subset of patients further substantiates our concept that left ventricular dysfunction is virus induced and can be partially reversed.

Conclusions. The present results favor the view that enterovirus-associated left ventricular dysfunction is a distinct disease entity with a benign course undetectable by clinical, anamnestic or hemodynamic features from idiopathic dilated cardiomyopathy. Detection of enterovirus infection in a subgroup of idiopathic dilated cardiomyopathy patients further extends our understanding of the pathogenesis of this multifactorial disease.

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