Role of hepatitis C virus in myocarditis and cardiomyopathies

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Abstract Recent nationwide clinic-epidemiological surveys in Japan showed that the occurrence of cardiomyopathies was most frequently seen in the age of sixties, and that cardiomyopathies are important causes of heart failure in the elderly. Viral infection was conventionally considered to cause myocarditis, which resulted in the development of dilated cardiomyopathy. Recent studies suggest that hepatitis C virus (HCV) is involved in the development of dilated cardiomyopathy, hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy in addition to myocarditis. Furthermore, left ventricular aneurysm represents the same morbid state not only after myocardial infarction but also after myocarditis. There were wide variations in the frequency of detection of HCV genomes in cardiomyopathy in different regions and in different populations. Major histocompatibility complex class II genes may play a role in the susceptibility to HCV infection, and may influence the development of different phenotypes of cardiomyopathy. If in fact the myocardial damage is caused by HCV, it might be expected that interferon (IFN) administration would be useful for its treatment. Hepatitis patients receiving IFN treatment for hepatitis were screened by thallium myocardial scintigraphy, and an abnormality was discovered in half of the patients. Treatment with IFN resulted in a disappearance of the image abnormality. It has thus been suggested that mild myocarditis and myocardial damage may be cured with IFN. We have recently found that high concentrations of circulating cardiac troponin T are a specific marker of cardiac involvement in HCV infection. By measuring cardiac troponin T in patients with HCV infection, the prevalence of cardiac involvement in HCV infection will be clarified. We are proposing a collaborative work on a global network on myocarditis cardiomyopathies due to HCV infection. (J Geriatr Cardiol 2004;1(2):83-89.)

Key Words myocarditis; cardiomyopathy; hepatitis C virus; hypertension; heart failure; interferon; major histocompatibility complex

Introduction Heart failure, often the consequence of cardiomyopathic disorders, is a major health concern in the developed countries. Cardiomyopathies may present itself as idiopathic dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), or restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and several other distinct disorders of the myocardium. DCM, HCM and RCM are heterogeneous myocardial disorders of multifactorial etiologies, including genetic anomalies and acquired immune pathogenetic factors, such as viral infections. DCM is a relatively common myocardial disorder, which may lead to severe heart failure. Along with ischemic heart disease it represents the main antecedent of heart transplantation in Western countries, where epidemiologic studies performed a decade ago, have measured 5-year survival rates as low as 30 to 40% after its initial diagnosis. In contrast, few large-scale studies have been conducted to examine the prevalence, prognosis and management patterns of cardiomyopathies in Asian populations.

Importance of cardiomyopathies in the elderly Recently, nationwide clinic-epidemiological surveys of cardiomyopathies were performed in Japan. Disorders surveyed included DCM, HCM, RCM, ARVC, mitochondrial disease, Fabry’s disease of the heart and prolonged Q-T interval syndrome. The total number of patients was estimated at 17 700 for DCM, 21 900 for HCM, 300 for RCM, 520 for ARVD, 640 for mitochondrial disease, 150 for Fabry’s disease of the heart, and 1 000 for prolonged Q-T interval syndrome. The prevalence of DCM and HCM was higher in men than in women; the male-to-female ratios were 2.6 and 2.3 for DCM and HCM, respectively. Occurrence of cardiomyopathies was most frequent in the age of sixties both in DCM (Fig. 1) and in HCM (Fig. 2). Therefore, cardiomyopathies are important causes of
heart failure in the elderly patients.

Role of viruses in the pathogenesis of cardiomyopathies

The myocardium is involved in a wide range of viral infections. In some cases, myocarditis may be the primary disorder; in others, it may occur as part of a systemic disease. Myocarditis is thought to be most commonly caused by enteroviruses, particularly coxsackie virus B. However, in many cases, when myocarditis has been diagnosed on the basis of clinical characteristics, no definite confirmation of viral origin was obtained despite extensive laboratory investigations. The evidence is often only circumstantial and a direct, conclusive proof of cardiac involvement is not available.5-7 However, accumulating evidence links viral myocarditis with the eventual development of DCM.8-14

The clinical presentation of viral myocarditis is variable. When myocardial necrosis occurs diffusely, congestive heart failure develops and, later, DCM. If myocardial lesions are localized, a ventricular aneurysm may form.

When complicated with arrhythmias, myocarditis presents itself as ARVC.12 When myocardial necrosis is localized within the subendocardium, RCM may develop. While it has not been established that hypertrophic cardiomyopathy may be a complication of viral myocarditis, asymmetrical septal hypertrophy has, in fact, sometimes been observed in patients with myocarditis.15

The myocardium may be the target of several types of viral infections. Recently, the importance of hepatitis C virus (HCV) has been noted in patients with HCM, DCM, and myocarditis and other heart diseases (Fig. 3).2,16-28 In a collaborative research project of the Committees for the Study of Idiopathic Cardiomyopathy in Japan, HCV antibody was found in 74 of 697 patients (10.6%) with HCM and in 42 of 663 patients (6.3%) with DCM; this prevalence in patients with cardiomyopathies was significantly higher than in age-matched volunteer blood donors in Japan (2.4%).

Importance of HCV infection in the elderly

The global prevalence of HCV carriers is estimated to be 3% on average, ranging from 0.1 to 10% or more in different countries.29 In Europe, the overall prevalence is 1% with a north-south gradient, ranging from 0.5% in northern countries to 2% in Mediterranean countries. Recent studies have shown high prevalence in Eastern Europe, ranging from 0.7% to 5%. In Asia, such as Mongolia, Viet Nam, Myanmar, and China show high prevalence. In Africa, high prevalence is seen only in central region countries and Egypt.30 In North America, the prevalence is relatively low. In South America, high prevalence is seen in Brazil.31,32 The highest prevalence (10% or more) is seen in Mongolia, Egypt, Tanzania, Guinea, and Cameroon.29,32 There are reasons for the high prevalence of HCV carriers in each of these regions. For example, in Egypt, the use of parenteral antischistosomal therapy is thought to have contributed to a prevalence of HCV spreading by 22%.30 There are 170 million chronic HCV carriers throughout the world, of whom an estimated 2 million are in Japan, 2.7 million in the United State, 5 million in Western Europe.

Until relatively recently, blood transfusion posed a major risk of HCV infection in developed countries. The automated RNN-extraction technology improved blood-screening tests by the detection of anti-HCV infection.33 The average prevalence of HCV carriers in Japan is about 2%, with the number estimated at 2 million. According to the study of Yoshizawa, the age-specific prevalence of anti-HCV among blood donors are < 0.5%, 0.5-1%, 1-2%, 2-3%, 3-4%, and > 4% in individuals < 34 years, 35-44 years, 45-54 years, 55-59 years, 60-64
years, and > 65 years, respectively. These is a clear increase with age in the prevalence of anti-HCV, reaching its highest rate of 7% in individuals > 70 years. Therefore, HCV infection is an important issue for the elderly.

**HCV infection and cardiomyopathies**

We first evaluated 31 patients with cardiomyopathy and myocarditis by polymerase chain reaction (PCR) for the presence of RNA viruses such as enterovirus, cardiovirus, hepatitis A virus, human immunodeficiency viruses 1 and 2, human T-lymphocytic leukemia virus 1, influenza A and B viruses, and reovirus. We also evaluated patients with cardiomyopathy and myocarditis for DNA viruses such as adenovirus, cytomegalovirus, Epstein-Barr virus, hepatitis B virus, human herpesvirus 6, varicella-zoster virus, and herpes simplex virus types 1 and 2. However, in only one (3.2%) patient with dilated cardiomyopathy enterovirus RNA was detected and no other virus genomes were found. On the contrary, we found HCV RNA in 6 patients (19.4%) with dilated cardiomyopathy (Table 1).

**Table 1. PCR analysis for RNA viruses in the hearts of patients with myocarditis and cardiomyopathy (n = 31)**

| Virus                | Positive(n)(%)
|----------------------|----------------
| Cardiovirus          | 0              |
| Enterovirus(Coxsackievirus B) | 1(3.2%) |
| Hepatitis A          | 0              |
| Hepatitis C          | 6(19.4%)       |
| HIV 1 and 2          | 0              |
| HTLV-1               | 0              |
| Influenza A          | 0              |
| Influenza B          | 0              |
| Reovirus             | 0              |

**HCV infection and dilated cardiomyopathy**

Over a 10-year period, we identified 19 of 191 patients (9.9%) with dilated cardiomyopathy who had evidence of HCV infection on the basis of a positive immunoradiometric assay, whereas only 1 patient (2.5%) of those with ischemic heart disease was positive for the HCV antibody. The difference was statistically significant. None of the patients with HCV antibody had a history of intravenous drug use. Mildly elevated levels of serum aminotransferase were found in 10 patients. The primary findings at presentation were heart failure and cardiac arrhythmias. Of the 19 patients with HCV antibodies, 10 patients had HCV RNA in the serum, and all 6 patients had type 1b HCV.

HCV RNA was found in the heart of 8 patients. Negative strands of HCV RNA were detected in the heart of 2 patients. Because negative RNA molecules are considered to be intermediates in the replication of the HCV genome, it is supposed that HCV replicates in myocardial tissues.

**HCV infection and hypertrophic cardiomyopathy**

Over a 10-year period, 16 of 113 patients (14.1%) with HCM were identified who had evidence of HCV infection on the basis of positive HCV antibody. In contrast, prevalence of positive HCV antibody in voluntary blood donors in Japan was 2.41% in subjects 55 to 59 years of age. The difference was statistically significant. Of these 16 patients, none of the patients had a family history of hypertrophic cardiomyopathy. Seven patients had hepatoma, 4 patients had had blood transfusions, and mildly elevated serum amino- transferases were measured in 10 patients. Nine patients had spade-shaped deformities of the left ventricle with a ratio of apical thickness to middle anterior free wall thickness > 1.3, and were diagnosed as apical hypertrophic cardiomyopathy. None had angiographically visible coronary artery disease.

Histopathological studies showed mild to severe degrees of myocyte hypertrophy in the right or left ventricle, mild to moderate fibrosis, and mild cellular infiltration. Type 1b HCV RNA was detected in the serum of 7 patients. Quantitative analysis of HCV RNA showed that the copy number in the serum was $5.5 \times 10^5$ to $4 \times 10^6$ genomes/ml. HCV RNA was found in the biopsy specimens of 6 patients. Negative strands of hepatitis C virus RNA were found in the hearts of 2 patients. Analysis by fluorescent single-stand conformation polymorphism showed the presence of multiple clones in the sera of patients with hypertrophic cardiomyopathy.

**HCV infection and heart diseases: A multicenter study in Japan**

Positive HCV antibodies were detected in 650 of 11,967 patients (5.4%) seeking care at 5 university hospitals, a significantly higher prevalence than in volunteer blood donors. Of the cardiac abnormalities observed in these patients with positive HCV antibody, arrhythmias were the most frequent (21.5%). Electrocardiographic abnormalities were found in 130 of 349 tested patients (62.8%), most often in the form of arrhythmias or conduction disturbances. Echocardiographic examination suggested that HCV infection was associated with left ventricular hypertrophy in over one-half of the patients, left ventricular dilation in 40%, and decreased left ventricular systolic function in 34%.

The study suggests that several cardiac abnormalities other than cardiomyopathic disorders (e.g. arrhythmias) may result from HCV infection, which may be a risk factor for some conditions (hypertension, myocardial infarction, etc., Table 2), although further study is necessary to con-
firm these associations. More recently, a possible role of HCV infection in the pathogenesis of atherosclerosis has been reported. 35

Table 2. Clinical diagnoses of 349 patients with HCV antibody seeking care at university hospitals

<table>
<thead>
<tr>
<th>Clinical diagnoses</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrhythmia</td>
<td>75</td>
<td>21.5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>71</td>
<td>20.3</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>57</td>
<td>16.3</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>50</td>
<td>14.3</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>45</td>
<td>12.9</td>
</tr>
<tr>
<td>Renal disease</td>
<td>41</td>
<td>11.7</td>
</tr>
<tr>
<td>Valvular heart disease</td>
<td>33</td>
<td>9.5</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>28</td>
<td>8.0</td>
</tr>
<tr>
<td>Hypertrophic cardiomyopathy</td>
<td>23</td>
<td>6.6</td>
</tr>
<tr>
<td>Post valvular replacement and/or CABG</td>
<td>22</td>
<td>6.3</td>
</tr>
<tr>
<td>Dilated cardiomyopathy</td>
<td>20</td>
<td>5.7</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7</td>
<td>2.0</td>
</tr>
<tr>
<td>Unclassified cardiomyopathy</td>
<td>2</td>
<td>0.6</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

HCV genomes from heart paraffin sections

A collaborative multicenter study was performed by the Scientific Council on Cardiomyopathies of the World Heart Federation (Bernhard Maisch, MD, Chairman) to test the reproducibility in the detection of viral genomes, such as enteroviruses, adenovirus, cytomegalovirus and HCV in formalin-fixed tissues. In this study, autopsy and biopsy materials were analyzed blindly. We found HCV genomes in 2 out of 11 (18%) of patients with DCM and myocarditis from Italy, and in 4 out of 11 (36%) from the United States, two were from patients with myocarditis, and the other 2 from patients with ARVC. These results suggest that HCV may cause ARVC as well as myocarditis, DCM and HCM. As the detection of HCV genomes in formalin-fixed sections seems less sensitive than in frozen sections, HCV infection may actually be a more prevalent cause of myocardial injury.

In a collaborative research project with the National Cardiovascular Center and Juntendo University, we tried detecting HCV genomes in paraffin sections of autopsied hearts. Among 106 hearts examined, β-actin gene was amplified in 61 (52.6%). Among these, HCV RNA was detected in 13 (21.3%), and negative strands in 4 hearts (6.6%). HCV RNA was found in 6 hearts (26.0%) with HCM, 3 hearts (11.5%) with DCM, and 4 hearts (33.3%) with myocarditis (Table 3). These HCV RNA positive samples were obtained between 1979 and 1990, indicating that HCV RNA can be amplified from paraffin-embedded hearts preserved for many years. 36

We also analyzed autopsied hearts with dilated cardiomyopathy from the University of Utah in a collaborative research, and found HCV RNA in 8 of 23 hearts (35%) with positive actin genes (Table 4). The sequences of HCV genomes recovered from these hearts were highly homologous to the standard strain of HCV. These observations lend support to the previous findings of an important role played by HCV in the pathogenesis of HCM and DCM.

Table 3. Detection of HCV genomes from the autopsied hearts of patients with myocarditis, and dilated and hypertrophic cardiomyopathies with positive β-actin gene

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Positive ratio</th>
<th>Percentage (%)</th>
<th>P (Fisher)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocarditis</td>
<td>4/12</td>
<td>33.3%</td>
<td>0.0008</td>
</tr>
<tr>
<td>DCM</td>
<td>3/26</td>
<td>11.5%</td>
<td>0.034</td>
</tr>
<tr>
<td>HCM</td>
<td>6/23</td>
<td>26.0%</td>
<td>0.0005</td>
</tr>
<tr>
<td>Myocarditis + DCM + HCM</td>
<td>13/61</td>
<td>21.3%</td>
<td>0.0002</td>
</tr>
<tr>
<td>Controls *</td>
<td>0/52</td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>

* IHD n = 32, Non-cardiac death n = 20

Table 4. Detection of HCV genome from the formalin-fixed paraffin sections of autopsied DCM hearts

<table>
<thead>
<tr>
<th></th>
<th>Positive HCV genomes/ Total DCM heart (%)</th>
<th>Positive HCV genomes/ Positive actin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Utah</td>
<td>18/72 (25%)</td>
<td>8/23 (34.8%)</td>
</tr>
<tr>
<td>LDS Hospital, Utah</td>
<td>0/31 (0%)</td>
<td>0/12 (0%)</td>
</tr>
<tr>
<td>St. Paul’s Hospital, Vancouver</td>
<td>0/24 (0%)</td>
<td>0/11 (0%)</td>
</tr>
<tr>
<td>Japan</td>
<td>5/50 (10%)</td>
<td>3/26 (11.5%)</td>
</tr>
</tbody>
</table>
However, there were wide variations in the frequency of detection of HCV genomes in cardiomopathy in different cities. HCV genomes were detected in none of 24 hearts from St. Paul’s Hospital, Vancouver, Canada. These results suggest that the frequency of cardiomopathy caused by HCV infection may be different in different regions or in different populations. Some European investigators have reported negative associations between HCV infection and DCM, though the disparity in results may be due to inappropriate controls, incomplete clinical investigation, or other factors such as regional or racial differences.

### Association of the genes of the major histocompatibility complex class II and cardiomopathies due to HCV infection

The human major histocompatibility complex (MHC) is located in the short arm of chromosome 6 and encodes several protein products involved in immune function. These include the complement, TNF-α, and the human leukocyte antigen (HLA) complex, whose polymorphisms are often proposed as candidates of susceptibility to various diseases. Associations of MHC class II antigens have been reported with patients with hypertrophic and dilated cardiomopathies. More recently, MHC class II genes have also been analyzed at the DNA level, though the results were inconsistent. In a Japanese study, the frequencies of DRB1 *1401, DQB1 *0503, and DRB1 *1101 were increased in patients with dilated cardiomopathy. However, the development of dilated cardiomopathy cannot be solely explained by the presence or absence of a single MHC class II allele. Since the etiology of dilated cardiomopathy is heterogeneous, different disease entities may be linked to different MHC class II genes.

Genetic studies to date have examined 3 aspects of HCV infection: 1) clearance, 2) progression (cirrhosis), and 3) susceptibility to infection. In studies on HCV hepatitis showed that DQB1 *0301 was associated with clearance of the virus. DRB1 *04 and DQA1 *03 were identified as protective alleles, which are in strong linkage disequilibrium with DQB1 *0301. DRB1 *1101, which is also in linkage disequilibrium with DQB1 *0301, was associated with clearance, and DRB1 *11 was associated in other studies. Several other studies have considered the association of MHC alleles with progression of liver disease. Two studies by Japanese compared HCV carriers with normal liver function tests and normal histology with patients with abnormal liver function tests and cirrhosis, respectively. In both studies, DQB1 *0401, DRB1 *0405, and two-locus haplotype consisting of these alleles were more frequent in those who developed chronic liver disease (Fig. 3).

### Therapeutic markers and HCV cardiomyopathies

In patients with HCV hepatitis, the success of treatment can be measured by the biochemical (normalization of alanine aminotransferase levels) and virologic (undetectability of serum HCV RNA) responses. However, therapeutic markers to follow HCV cardiomyopathies have not been established in clinical practice. We have examined the effect of interferon on myocardial injury associated with active HCV hepatitis in collaboration with Shimane University. Since TI-201-SPECT is a more sensitive method than electrocardiography or echocardiography to detect myocardial injury induced by HCV, we used TI-SPECT scores to measure the effects of interferon on myocardial injury. SPECT scores improved in 8 out of 15 patients (53%) whose interferon treatment was completed. Circulating HCV disappeared after interferon therapy in all 11 patients, with either a decrease or no change in SPECT scores, but HCV genomes persisted in the blood circulation of 2 patients.
whose clinical status worsened. This preliminary study suggests that interferon is a promising treatment for myocardial diseases caused by HCV infection.

We have recently reported that patients with DCM whose prognosis is poor have abnormally high serum concentrations of cardiac troponin T in the absence of an increase in serum creatine kinase concentrations, and in that population, cardiac troponin T is a prognostic marker. Serial measurements of serum cardiac troponin T concentrations seem to be a reliable indicator of myocyte injury, and we have hypothesized that, in patients with cardiomyopathy, therapeutic interventions for heart failure which ultimately improve the prognosis should be associated with a fall in cardiac troponin T. Therefore, in patients with HCV cardiomyopathies monitoring of HCV RNA and cardiac troponin T appears appropriate.

We have reported the treatment with interferon for an elderly patient with DCM and striated myopathy associated with HCV infection, guided by serial measurements of serum HCV RNA and cardiac troponin T for the first time. In that patient, serum concentrations of cardiac troponin T remained abnormally high over a 3-year period despite treatment of heart failure with angiotensin-converting enzyme inhibitors, β-adrenergic blockers, calcium antagonists, dopamine, and dobutamine. Clinical manifestations of heart failure progressed, while echocardiographic left ventricular ejection fraction decreased from 49% to 29%, and left ventricular end-diastolic dimension increased from 60 mm to 69 mm. HCV RNA in heart tissue was positive by PCR. Interferon therapy was introduced with monitoring of cardiac troponin T concentration, which fell in parallel with a decline in serum HCV RNA during treatment. It is also noteworthy that, after cessation of interferon therapy, serum concentrations of cardiac troponin T and serum HCV RNA returned toward their baseline values (Fig. 5, left).26 These observations strongly suggest that the myocyte injury documented in this patient was related to HCV infection. We have now treated another patient with HCV cardiomyopathy with interferon and, both the HCV RNA and cardiac troponin T of this patient fell concomitantly during treatment (Fig. 5, right).

We are proposing a collaborative work on a global network on myocarditis cardiomyopathies to clarify the prevalence of cardiac involvement in HCV infection, and to perform treatment trials.

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