

# Cryoglobulinaemia and rheumatic manifestations in patients with hepatitis C virus infection

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## Abstract

**Objectives**—To investigate the association of cryoglobulinaemia and rheumatic manifestations in Korean patients with hepatitis C virus (HCV) infection.

**Methods**—Forty nine Korean patients with HCV infection were recruited. The prevalence, concentration, and type of cryoglobulin (by immunofixation), rheumatoid factor (RF), antinuclear antibody (ANA), and various rheumatological symptoms were investigated and HCV genotype was determined by polymerase chain reaction with genotype specific primer.

**Results**—The prevalence of cryoglobulin was 59% in Korean HCV patients and the concentration of cryoglobulin was 9.8 (7.9 g/l (mean (SD))). The type of cryoglobulinaemia was identified in 23 (80%) of 29 HCV patients with cryoglobulinaemia and they were all type III. There were no differences in age, sex, history of operation and transfusion, proportion of liver cirrhosis between the patients with cryoglobulinaemia and those without cryoglobulinaemia. The frequencies of RF and ANA were 14% and 3.4% respectively in HCV patients with cryoglobulinaemia. There was no difference in HCV genotype between the patients with cryoglobulinaemia and those without cryoglobulinaemia. Clinical features of HCV patients were as follows: arthralgia/arthritis (35%), cutaneous manifestation (37%), Raynaud's phenomenon (8%), paresthesia (44%), dry eyes (22%), dry mouth (10%), oral ulcer (33%), and abdominal pain (14%). However, these rheumatological symptoms did not differ between the two groups.

**Conclusion**—Although the rheumatological symptoms were not different between HCV patients with and without cryoglobulinaemia, HCV patients showed various rheumatological manifestations. These result suggests that HCV infection could be included as one of the causes in patients with unexplained rheumatological symptoms.

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Hepatitis C virus (HCV) is the major cause of post-transfusion and sporadic non-A, non-B chronic hepatitis worldwide.<sup>1</sup>The worldwide prevalence of HCV infection varies. In Korea, seroprevalences of anti-HCV antibodies are estimated to be about 0.5 to 0.9% for blood donors<sup>2</sup> and 1.3% to 1.7% for Korean adults

undergoing a general check up<sup>3</sup> as compared with about 1% seroprevalence of HCV in many developed countries.<sup>4</sup>

It has been shown that HCV infection might be associated with mixed cryoglobulinaemia.<sup>5-7</sup> Cryoglobulins are proteins that reversibly precipitate from blood on cooling and they are classified on the basis of their immunoglobulin (Ig) composition according to Brouet *et al*<sup>8</sup>; type I consists of monoclonal Igs, type II is a mixture of monoclonal and polyclonal Igs, and type III consists of polyclonal Igs. Cryoglobulins are often found in association with collagen-vascular diseases, lymphoproliferative diseases, and several infectious diseases including HCV infection.<sup>8-9</sup> It has been also reported that HCV infection may be associated with a broad spectrum of extrahepatic diseases such as membranoproliferative glomerulonephritis, Sjögren's syndrome, rheumatoid arthritis, inflammatory arthritis, peripheral neuropathy, and cutaneous manifestations.<sup>10-15</sup> But the prevalence and clinical significance of cryoglobulin remain unclear in patients with HCV infection. The role of HCV infection in the rheumatic diseases continues to be of interest to rheumatologists.

This is the first study in Korea to evaluate the link of cryoglobulinaemia and rheumatological symptoms in patients with HCV infection. We therefore examined the prevalence and type of cryoglobulinaemia and various rheumatic manifestations in Korean patients with HCV infection.

## Methods

### PATIENTS

Forty nine Korean patients (27 male, 22 female) with HCV infection were selected for the study. They were randomly recruited from the hepatology clinic of Guro Hospital, Korea University between August 1996 and July 1997. They all gave verbal consent to this study. In all cases, the diagnosis of chronic hepatitis C was based on the following criteria: (1) the presence of serum alanine aminotransferase (ALT) activities more than twice the upper limit of normal range, (2) positive serological markers of HCV infection such as antibodies to hepatitis C by enzyme immunoassay kit (Abbott Laboratories, North Chicago, Ill, USA) and hepatitis C RNA PCR that was carried out using nested primers specific for the 5' untranslated region as previously described,<sup>16</sup> and (3) the absence of any other cause of chronic liver disease.

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Table 1 Clinical and laboratory findings in 49 HCV patients with and without cryoglobulinaemia

	Cryoglobulin positive (n=29)	Cryoglobulin negative (n=20)	p Value
Age (y)	59 (12)	54 (11)	NS
Sex (M/F)	14/15	13/7	NS
Operation	12 (41%)	11 (55%)	NS
Transfusion	8 (28%)	7 (35%)	NS
ALT (IU/L)	69 (42)	57 (37)	NS
CH:LC	17:12	13:7	NS
RF	4 (14%)	0	NS
ANA	1 (3.4%)	0	NS
HCV-RNA genotype (Ib/IIa)*	8/2	4/5	NS

Mean (SD), CH: chronic hepatitis, LC: liver cirrhosis, \*: Simmonds' classification, NS: not significant.

#### DETECTION, ISOLATION, AND CHARACTERISATION OF CRYOGLOBULINS

Ten millilitres of blood were taken from the patients into anticoagulant free tubes by using pre-warmed equipment and were maintained at 37°C until coagulation was complete. Serum was isolated by centrifugation (2000 × *g* for 30 minutes) at 37°C and stored at 4°C for eight days. We checked the reversible nature of the precipitate. Cryoglobulins were isolated by centrifugation (2000 × *g* for 15 minutes) and washed five times in a minimal volume of 0.15 mol/l sodium chloride. All steps were carried out at 4°C. A fraction of the washed cryoglobulins was diluted in 0.1 mol/l NaOH, and the absorbance was read in a spectrophotometer at 280 nm. The results were expressed relative to a standard curve derived by using a purified human  $\gamma$ -globulin preparation (Sigma, St Louis, MO).<sup>17</sup> Characterisation of cryoglobulins was performed using a commercially available immunofixation electrophoresis kit (Ciba Corning, San Antoni, CA), and cryoglobulins were classified on the basis of their Ig composition into three types according to Brouet *et al.*<sup>8</sup>

#### CLINICAL DATA

All patients received biochemical tests to measure serum activities of ALT, serum alkaline phosphatase activities, serum bilirubin values, and urine analyses. All patients were evaluated for antinuclear antibody (ANA) using indirect immunofluorescence of HEP-2 cells and rheumatoid factor (RF) using nephelometry. The HCV genotype was studied in 19 patients by amplification of the core region of HCV using PCR according to the method described by Okamoto *et al.*<sup>18</sup> According to Simmonds' classification,<sup>19</sup> Okamoto's HCV II was described as genotype Ib, HCV III as genotype 2a, and HCV IV as genotype 2b.

All patients were questioned for history of transfusion and operation, the presence of rheumatological symptoms including arthralgia/arthritis, Raynaud's phenomenon, paresthesia, dry eyes, dry mouth, oral ulcer, abdominal pain, and cutaneous manifestation such as rash.

#### STATISTICAL ANALYSIS

Data were expressed as mean (SD). Statistical analysis was performed using  $\chi^2$  test and Fisher's exact test. Differences were considered to be significant when p value was less than 0.05.

## Results

### PATIENTS CHARACTERISTICS

Table 1 shows the characteristics of the 49 HCV patients with and without cryoglobulinaemia. The mean age was 56.9 years. The mean age, sex, ALT, history of both transfusion and operation were not significantly different between the two groups. Cirrhosis was present in 12 of 29(41%) and 7 of 20(35%) of cryoglobulinaemia positive and cryoglobulinaemia negative groups, respectively. But there was no statistical difference between these two groups ( $p > 0.05$ ) (table 1).

### CRYOGLOBULINAEMIA

Cryoglobulin was present in 29 of the 49 (59%) HCV patients. The mean concentration of cryoglobulins was 9.8 (7.9) g/l. The type of cryoglobulinaemia was identified in 23 cases (80%) among the 29 HCV cryoglobulin positive patients by immunofixation electrophoresis and they were all type III cryoglobulinaemia. RF and ANA were detected in four (8.1%) and one (0.2%) of HCV patients, respectively. They were present in four (14%) and one (3.4%) of the 29 HCV patients with cryoglobulinaemia, respectively, but not in the 20 patients without cryoglobulinaemia. However, there was no statistical difference ( $p > 0.05$ ). When present, the titre of RF and ANA was usually low (RF < 50 IU/ml, ANA < 1:80), and the ANA pattern was speckled.

HCV-RNA genotype was evaluated in 10 of the 29 HCV cryoglobulin positive patients and in nine of the 20 HCV cryoglobulin negative patients. Among the 10 HCV cryoglobulin positive patients examined, there were eight and two patients carrying Ib and IIa, respectively. Among the nine HCV cryoglobulin negative patients examined, four and five patients carried HCV-RNA genotype Ib and IIa, respectively. There was no significant difference in HCV-RNA genotype between the two groups ( $p > 0.05$ ) (table 1).

### RHEUMATOLOGICAL SYMPTOMS

Rheumatological symptoms were assessed by questionnaire. Table 2 shows the rheumatic manifestations of the HCV patients. They were; 35% with arthralgia/arthritis, 37% with cutaneous manifestation(rash), 8% with Raynaud's phenomenon, 44% with paresthesia, 22% with dry eyes, 10% with dry mouth, 33% with oral ulcer, and 14% with abdominal pain. The arthralgia/arthritis they expressed was of no specific pattern and was not associated with morning stiffness. We examined the patients with cutaneous manifestation, but cutaneous manifestation was non-specific and did not suggest vasculitis. There was also no other evidence suggestive of vasculitis in the patients and no vasculitis was found in any of our patients.

No patient, including those who were RF or ANA positive, had a rheumatological disorder that satisfied American College of Rheumatology (ACR) criteria and the rheumatological symptoms were not different between the HCV patients with and without cryoglobulinaemia ( $p > 0.05$ ) (table 2).

Table 2 Rheumatological symptoms in 49 HCV patients with and without cryoglobulinaemia

	Cryoglobulin positive (n=29)	Cryoglobulin negative (n=20)	p Value
Arthralgia/arthritis	31	40	NS
Raynaud's phenomenon	10	5	NS
Paresthesia	41	50	NS
Dry eyes	24	20	NS
Dry mouth	14	5	NS
Oral ulcer	28	40	NS
Abdominal pain	10	20	NS
Cutaneous manifestation (rash)	31	45	NS

Data shown as percentages.

### Discussion

It has been reported that HCV infection was implicated in mixed cryoglobulinaemia and associated with various rheumatic manifestations.<sup>10-15</sup> Although various rheumatological manifestations can be the presenting complaint of HCV infection, the prevalence and clinical significance of cryoglobulin in patients with HCV infection remains unclear. Several studies on patients with HCV infection reported the prevalence of cryoglobulinaemia, varying from 13% to 56%.<sup>20-21</sup> HCV seemed to trigger autoimmune responses that might be manifested as autoantibodies, cryoglobulin, or extrahepatic manifestations.<sup>11-12, 22</sup> Autoantibodies such as RF or ANA occur with HCV infection, usually in low titres. It has been reported that 36% to 76% of RF and 10% to 30% of ANA were present in HCV patients.<sup>17-23</sup> The mechanisms leading to the formation of autoantibodies after HCV infection remain obscure, but direct infection of lymphocytes by HCV may play a part,<sup>23-25</sup> and chronic immune complex stimulation could influence autoantibodies production.<sup>26-27</sup>

In our study, the prevalence of cryoglobulin was 59% in Korean patients with HCV infection as compared with 37% in Japanese HCV patients<sup>10</sup> and all of the cryoglobulins identified in our study belonged to type III. Of course these data do not mean that all Korean cryoglobulins are type III. A portion of cryoglobulins in cases unidentified by immunofixation can be type II. HCV patients showed various rheumatological symptoms, but there was no specific rheumatological disease that fulfilled ACR diagnostic criteria. Neither the rheumatological symptoms nor the HCV genotypes showed statistical difference between the HCV patients with and those without cryoglobulinaemia. The prevalence of cirrhosis was similar in HCV infected patients with and without cryoglobulins in contrast with the findings of Lunel *et al*<sup>21</sup> who reported increased cirrhosis among the HCV patients with cryoglobulins. Although the prevalence of cirrhosis did not reach statistical significance, it showed a higher tendency in HCV patients with cryoglobulin than in the patients without cryoglobulin.

RF and ANA were present in low frequency. They were present in four (14%) and one (3.4%) of the 29 HCV patients with cryoglobulinaemia, respectively, and were not in the 20 patients without cryoglobulinaemia. The frequency of RF and ANA was 50% and 32.3%, respectively in Japanese cryoglobulin positive

HCV patients, and was 33.3% and 28.1%, respectively in Japanese cryoglobulin negative patients.<sup>17</sup> The low prevalence of RF in our patients with cryoglobulins is surprising. However, the more relevant RF determinations are those on the cryoglobulins because the immune complexes involved in the vasculitis associated with cryoglobulinaemia are the RF containing mixed cryoglobulins as originally described by Meltzer *et al*.<sup>28</sup> Although we did not determine RF activity of the cryoglobulins, we think that study on the RF activity is needed. The exact reason for the lower frequency of RF and ANA in our study than those in Japanese<sup>17</sup> and Western study<sup>23</sup> was not clearly understood, but there can be the possibility of different genetic, environmental, and virological factors between Western, Japanese, and Korean HCV patients and the possibility that some of the patients may have hidden RFs. Our finding on cryoglobulin types in HCV patients was similar to those reported by Kerr *et al*<sup>29</sup> and Japanese studies<sup>17</sup> that cryoglobulin was often of the type III, but the result on the occurrence of rheumatological symptoms was different from results obtained by Kerr *et al*<sup>29</sup> that rheumatological symptoms occurred in HCV cryoglobulinaemia positive patients more than in HCV cryoglobulinaemia negative patients. This difference may be because of relatively small subject numbers in our study, but the possibility that rheumatological symptoms can occur independently of cryoglobulinaemia in HCV patients cannot be excluded.

In this study, the absence of specific rheumatological disease in HCV patients may be explained by several factors as Kerr *et al*<sup>29</sup> suggested. Firstly, the numbers in this study may have been too small to detect the disorders. Secondly, the duration of HCV infection may be of importance for the development of mixed cryoglobulinaemia and patients with longer periods of infection may be more likely to develop symptoms<sup>20</sup> but unfortunately it is very difficult to establish onset of HCV infection, thus the disease duration generally cannot be defined. Thirdly, the majority of patients with essential mixed cryoglobulinaemia have type II cryoglobulins.<sup>30</sup> In contrast, cryoglobulins identified by immunofixation were type III in our study.

In Western Europe and the United States, HCV infection is predominantly caused by genotypes 1a, 1b, 2b, and 3a, with some variation in frequency.<sup>16-18</sup> And in Japan and Taiwan, genotypes 1b, 2a, and 2b are seen most frequently.<sup>31-32</sup> Although the HCV genotypes in Korean patients were not well studied, genotypes 1b and 2a were seen frequently in our study and this result was similar to the result of Japanese HCV patients.<sup>17</sup> The role of HCV genotypes in mixed cryoglobulinaemia is unclear, but cryoglobulin seems to be independent on HCV genotype.<sup>10-22, 32</sup> Distribution of the viral genotype did not differ in both of the groups we examined.

We measured HCV-RNA concentration by quantitative PCR and compared HCV-RNA concentration in cryoprecipitates with that in supernatants. We also examined the prevalence

of cryoglobulinaemia in patients with hepatitis B virus infection. Although there was no statistical difference, HCV-RNA concentration was higher in HCV cryoglobulinaemia positive patients than in HCV cryoglobulinaemia negative patients (10 (5) compared with 10 (4) copies/ml,  $p > 0.05$ ) (data not shown). HCV-RNA was concentrated 10 to 100 times in cryoprecipitates more than in supernatants of the selected four HCV patients (data not shown). Cryoglobulin was present in three of the 23 (13%) patients with hepatitis B virus infection (data not shown). These data suggest that HCV infection can be implicated in cryoglobulinaemia.

In conclusion, the prevalence of cryoglobulinaemia in Korean HCV patients was similar to results from other studies. There was no specific rheumatic disease that developed in our HCV patients. Although the rheumatological manifestations were also not different between the HCV patients with and those without cryoglobulinaemia, HCV patients showed various rheumatological symptoms. This study suggests that HCV infection should be considered as one of the causes in patients with unexplained rheumatological symptoms.

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