Heterogeneity of Hepatitis C Virus Genotypes in Hemophilia: Relationship With Chronic Liver Disease

By F.E. Preston, L.M. Jarvis, M. Makris, L. Philp, J.C.E. Underwood, C.A. Ludlam, and P. Simmonds

In this study we have determined the hepatitis C virus (HCV) serotype and genotype in a cohort of 96 HCV-infected hemophiliacs and have examined the relationship between HCV genotype and severity of chronic liver disease as determined by liver biopsy. HCV serotype was determined by specific enzyme-linked immunosorbent assays (ELISAs) and genotype by restriction fragment length polymorphism (RFLP) and HCV viral sequencing. The pattern of genotype distribution was quite unlike that of HCV-infected United Kingdom (UK) blood donors in that five of the six known HCV genotypes were represented, 50% were type 1, 13% type 2, and 18% type 3. An unexpected observation was the presence of HCV genotype 4 in four patients and type 5 in two patients. An additional feature was the presence of mixed infection, detected in 14% and 7% by serotype and genotype analysis, respectively. Liver biopsies were available from 51 patients. Cirrhosis was present in five of 27 (19%) of individuals with type 1, in 2 of 9 (22%) with type 2, and 5 of 8 (63%) of those with type 3. The heterogeneous pattern of HCV genotype distribution in this cohort of patients and the observed relationship between the severity of the related liver disease and specific HCV genotype may have important implications with respect to the natural history and treatment of HCV-related chronic liver disease in infected hemophiliacs worldwide.

© 1995 by The American Society of Hematology.
The cleavage patterns in the bation carried out at 4°C overnight. Statistics described with the exception that DNA was purified using Magic HinjI," HCV Typing type 1, 25% of type 2, and in only one patient with HCV type 3. Intermittently abnormal results were seen in 27% of PCR negative patients. Of the other three PCR negative subjects, persistently abnormal liver enzymes were seen in 14%. In 14% of individuals with type 1, 25% of type 2, and 5% of type 3. Mixed serotypes were demonstrated in 14%. In 19 cases (20%), the serotype could not be determined by ELISA.

Liver Function Tests
Persistently abnormal ALT levels were observed in 56% of HCV genotype 1 patients, 75% of type 2, and 88% of type 3. Intermittently abnormal results were seen in 27% of type 1, 25% of type 2, and in only one patient with HCV genotype 3. Normal ALT levels were observed in 17% of type 1 patients, in a single patient only with type 3, and in three of six PCR negative patients. Of the other three PCR negative subjects, persistently abnormal liver enzymes were seen in two, and intermittent abnormalities in one.

Liver Histology
Biopsy specimens from 28 patients were classified as chronic persistent hepatitis, 10 as chronic active hepatitis, and 13 as cirrhosis. One patient who was PCR negative had chronic persistent hepatitis.

HCV Serotypes
Results of the HCV typing studies are presented in Table 1. Of the 96 hemophiliacs tested, 59% were serotype 1, 2% type 2, and 5% type 3. Mixed serotypes were demonstrated in 14%. In 19 cases (20%), the serotype could not be determined by ELISA.

HCV Genotypes
As indicated in Table 1, there was considerable discordance between individual genotype and serotype, and identical results were observed in 44 of the 77 individuals in whom it was possible to serotype. On genotype analysis, 50% were type 1, 13% type 2, and 18% type 3. In addition, four patients were type 4 and two type 5. Seven patients had a mixed infection by this method (Table 1). Mixed infections and HCV genotypes 4 and 5 were observed only in patients who had received commercially derived products. We also examined the relationship between HCV genotype and human immunodeficiency virus (HIV) antibody status. The incidence of coinfection with HIV among the three HCV genotypes 1, 2, and 3 was 27%, 25%, and 35%, respectively.

In view of the observed discrepancies between HCV serotype and genotype, HCV virus sequencing was performed on samples from seven such patients. For all seven samples, identical results were obtained in the genotype derived from RFLP analysis and that from direct sequencing.

Relationship Between Liver Histology and HCV Genotype
Cirrhosis was present in 5 of 27 (19%) biopsy specimens of individuals with type 1; in 2 of 9 (22%) individuals with type 2, and in 5 of 8 (63%) with type 3 (Table 2). The incidence of cirrhosis was significantly greater with type 3 compared with non-type 3 HCV (5 of 8 v 8 of 34) [P < .05]. In patients with genotype 1, cirrhosis was documented on biopsy at 12, 15, 20, and 21 years following the first exposure to pooled clotting factor concentrates, compared with patients with genotype 3, where it was found after 3, 4, 6, 18, and 20 years. There were no significant differences between the HCV groups with respect to duration of HCV infection from initial exposure, age, or in type or severity of hemostatic defect (results not given). In addition, there was no apparent relationship between coexistent HIV infection and the presence of hepatic cirrhosis. Two patients were coinfected with the hepatitis B virus; in one the HCV genotype was 1 and histology chronic persistent hepatitis (CPH), while in the other the HCV genotype was 2 + 3 and the histology chronic active hepatitis (CAH). In one patient with genotype 2 HCV, alcohol abuse was believed to have played a contributory role in the development of cirrhosis.

DISCUSSION
The transmission of HCV to hemophiliacs by clotting factor concentrates in the period before the introduction of effective viral inactivation procedures is well established. This occurred largely as a consequence of the very large donor
of these HCV genotypes show highly restricted geographic distributions in that type 4 has been reported from the Middle East and Zaire,6,7,18 and type 5 occurs predominantly in South Africa,6 although recent reports indicate that it is present in low frequency in The Netherlands,19 Australia,15,16 and Canada.16 Taken together, these two HCV genotypes comprise 6.3% of the infective HCV agent in our patients. Therefore, it seems highly likely that this is a reflection of the source of the donor pool. Because our patients have received a number of different clotting factor concentrates, we are unable to relate the transmission of these HCV genotypes to any particular product. However, infection with HCV genotypes 4 and 5 was confined to patients with hemophilia A. Although the numbers are small, it is possible that commercial products were responsible for the transmission of these genotypes because hemophilia B patients were treated exclusively with UK-derived material, and these particular genotypes have not been identified in UK donors.

In this cohort of hemophiliacs, some differences were observed between HCV serotype and genotype. This is in contrast to the situation in HCV-infected blood donors in whom there is close agreement between the two assays. The observed differences in hemophiliacs is likely to relate to their reinfection with different HCV types, which could differ in their replication rates and in their capacity to induce HCV antibody formation. The recent observation that changes in the major circulating HCV genotype can occur in some infected hemophiliacs (Simmonds, unpublished observation) may also provide some explanation for the observed discrepancies between HCV serotype and genotype in our patients.

Simmonds et al7 have suggested that the degree of sequence variability of HCV is sufficient to significantly alter the antigenic and biologic properties of the virus, and they have demonstrated that in HCV antibody positive Scottish blood donors alanine aminotransferase levels are greater in individuals infected with HCV type 3 than in those with the more common type 1 infection. Other groups have also reported possible differences in the natural history of different HCV serotypes and also in their responsiveness to interferon,20-22 but the adoption of different HCV classification systems by the various groups makes comparisons somewhat difficult.

We have confirmed previous suggestions that HCV genotype 3 is associated with a more aggressive form of chronic liver disease than that associated with types 1 and 2.10 Because there were no differences between the HCV genotype groups with respect to duration of exposure, coexistent HIV infection, age, and severity of hemophilia, we conclude that this effect is probably HCV-type specific. It was interesting that in three patients with type 3 HCV cirrhosis developed within 3 years of exposure to concentrates compared to patients with type 1, where all cases developed after 12 years.

Other groups have suggested severe liver disease occurs in relation to type 1 infections,22 especially type 1b.20 However, it should be appreciated that the natural history of HCV infection in hemophiliacs may differ from that of other groups because there are important differences with respect to viral load, mixed HCV genotype infections, and immune suppression associated with clotting factor concentrates. The emergence of a relationship between a particular HCV geno-

### Table 2. HCV Genotypes in Relation to Liver Histology

<table>
<thead>
<tr>
<th>HCV Genotype</th>
<th>CPH No. (%)</th>
<th>CAH No. (%)</th>
<th>Cirrhosis No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 (56)</td>
<td>7 (26)</td>
<td>9 (19)</td>
</tr>
<tr>
<td>2</td>
<td>4 (44)</td>
<td>3 (33)</td>
<td>2 (23)</td>
</tr>
<tr>
<td>3</td>
<td>3 (38)</td>
<td>0</td>
<td>5 (63)</td>
</tr>
<tr>
<td>4</td>
<td>2 (66)</td>
<td>0</td>
<td>1 (33)</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 + 2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 + 3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative PCR</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Percentage within each genotype.
type and progressive liver disease is highly complex and undoubtedly reflects a number of interrelated factors affecting virus-host interactions.

There is increasing evidence that the natural history of HCV-related liver disease may be greatly influenced by HCV genotype. The global distribution of a large number of genotypes and subtypes of HCV has resulted in a complex pattern of HCV infection in hemophiliacs treated with pooled plasma-derived products. In view of the growing evidence that the eventual clinical outcome, including interferon responsiveness, of HCV infection may be influenced by HCV genotype, it seems clear that this will have considerable impact on the natural history of HCV-related chronic liver disease in hemophiliacs in different parts of the world.

REFERENCES


Heterogeneity of hepatitis C virus genotypes in hemophilia: relationship with chronic liver disease

FE Preston, LM Jarvis, M Makris, L Philp, JC Underwood, CA Ludlam and P Simmonds