

Viral clearance occurs very early during the natural resolution of hepatitis C virus infection in persons with haemophilia

M. E. EYSTER,* J. SANDERS* and J. J. GOEDERT†

*Division of Hematology/Oncology, Department of Medicine, The Pennsylvania State University College of Medicine, Hershey, PA; and †Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA

Summary. We studied spontaneous hepatitis C virus (HCV) RNA clearance in 12 haemophilic patients. In their earliest anti-HCV positive samples, HCV RNA was undetectable in eight patients (66%), positive by polymerase chain reaction (PCR) but negative by branched-DNA (bDNA) in three others, and quantifiable by bDNA (4839 IU/mL) in only one patient. In contrast, in earliest anti-HCV positive samples from eight matched controls who had persistent viremia, HCV RNA was quantifiable by bDNA in seven ($P = 0.0008$) and at higher levels (range 4644–678 515 IU/mL; median 43 532 IU/mL).

From initial HCV infection, HCV RNA cleared in 7 months or less in four patients and in 1–2 years in six others. HCV persisted for 5 years before clearance in the absence of repeated exposure in one patient. We conclude that HCV clearance usually but not always occurs within 1–2 years after infection and is more likely in those with lower than in those with higher early viral loads.

Keywords: haemophilia, hepatitis C, spontaneous hepatitis C virus RNA clearance

Introduction

Serological and virological data from several large cohort studies imply that between 15% and 46% of persons who acquire hepatitis C virus (HCV) infections recover and become HCV RNA-negative [1–8]. Limited clinical observations suggest that most cases of spontaneous resolution occur within the first 6 months to 1 year following acute infection [9–14]. However, the factors affecting viral clearance are not well defined, and it is unknown how long virus can persist before clearance in a person not repeatedly exposed.

Nearly all haemophiliacs who were infused with clotting factor concentrates before the introduction of donor screening and viral inactivation techniques in the mid-1980s were infected with the HCV [15–17]. In this study, we examined the timing of spontaneous viral clearance and its relationship to initial HCV RNA viral loads in a well-characterized cohort of haemophilic patients who were infected with HCV through contaminated plasma prior to 1988.

Materials and methods

From a cohort of 100 haemophiliacs who were repeatedly exposed to unsterilized blood products and followed in our comprehensive care clinic at intervals of 6 months to 1 year, we previously identified 14 HIV-negative, anti-HCV positive patients who were never treated with interferon- α and had no detectable HCV RNA. [18]. In this report, we tested archived sera from 12 of these patients for the presence of HCV RNA. Initial testing was performed on the first available samples. Subsequent samples were tested sequentially, based on

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Correspondence: M. Elaine Eyster MD, Penn State Hershey Medical Center, PO Box 850, MC 46 Hershey, PA 17033, USA. Tel.: +717-531-8399; fax: +717-531-0647; e-mail: eeyster@psu.edu

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Table 1. Demographic data on 12 patients with viral clearance.

Subject age/sex deficiency	Month last anti-HCV negative or first year transfusion	Date first HCV-positive	Age at infection (years)*
1. 33F FIX	April 1981	February 1985	15
2. 27 F VWF	1978	September 1980	5
3. 60 M FVIII	June 1978	June 1980	38
4. 20 M FV	October 1985	December 1987	5
5. 18 M FVIII	June 1985	November 1986	2.5
6. 19 M FVIII	March 1984	October 1985	2.5
7. 19 M FVIII	February 1986	September 1986	4
8. 27 M FVIII	1975	December 1979	3
9. 74 M FVIII	April 1981	November 1981	54
10. 16 M FVIII	1985	September 1987	1
11. 32 M FVIII	December 1977	March 1978	8.5
12. 44 M FVIII	March 1977	May 1977	20

*Calculated from the midpoint between the maximum and the minimum duration of infection (see Methods).
HCV, hepatitis C virus; VWF, von Willebrand factor.

availability. A total of 97 samples (median 6, range 3–10 samples per patient) collected during routine clinic visits from 1978 and 2002 were available for testing. All patients were followed with informed consent since 1973 and enrolled in the Multicenter Hemophilia Cohort Study since 1982. All were caucasian. Ten were male. Nine had classic haemophilia A, and one each had von Willebrand's disease, factor IX deficiency, and FV deficiency. Ages as of December 2001 ranged from 16 to 74 years (median 27 years) (Table 1).

Hepatitis C virus RNA assays were performed on archived samples collected from 1978 to 2002 and stored frozen without thawing until testing. Eight otherwise healthy haemophilic patients with persistent viraemia matched for age, sex and duration of blood product exposure served as controls. Anti-HCV testing was performed on first available samples with no detectable HCV RNA to validate infection and to define more precisely the timing of the initial infection. Earliest possible date of infection was defined as the later of the first blood product exposure and the last anti-HCV negative sample.

Latest possible date of infection was the earliest of the first use of unsterilized factor concentrates, and the first positive sample for anti-HCV or HCV RNA. Times of and ages at infection were estimated at the midpoint between the earliest and latest possible dates.

Hepatitis C virus RNA was detected in serum by polymerase chain reaction (PCR) with the COBAS Amplicor assay (Roche Diagnostic Systems, Indianapolis, IN, USA). Viral loads were quantified on PCR-positive samples with the Versant branched-DNA (bDNA) assay, ver. 3.0 (Bayer Diagnostics, Tarrytown, NY, USA). HCV genotyping was performed on PCR products with the InnoLiPA reverse hybridization assay (Bayer Diagnostics). HCV antibody testing was performed using the Abbott ver. 2.0 assay (Abbott Labs, Abbott Park, IL, USA) for those samples that had not been previously tested with a first generation enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostics System, Raritan, NJ, USA) and confirmed positive with a second-generation recombinant immunoblot assay (RIBA-2; Chiron Corp. Emeryville, CA, USA).

Results

We studied 12 patients with spontaneous HCV clearance, infected 12–24 years (median 19 years) earlier. The interval from the first possible infection date to the first positive test for anti-HCV or HCV RNA was 2–7 months in four patients, 1–2 years in six patients, and 4 years in two patients who lacked interim samples (Table 2). All had had asymptomatic primary HCV infections, except for one (patient no. 12) who was simultaneously co-infected with hepatitis B. Testing initial anti-HCV positive archived samples, we found that HCV RNA was cleared very early, usually within the first 1–2 years following the primary infection. HCV RNA was undetectable by PCR in the initial samples from eight (subjects nos 1–8) (66%) of the 12 patients (Table 2 and Fig. 1). In three other patients' initial samples (patients nos 9–11), HCV RNA was detectable by PCR but not by bDNA. HCV RNA was detected at a low level by bDNA (4839 IU/mL, cut off <615 IU/mL) in one patient's initial sample (patient no. 12). Eight of the 12 patients had been infected with HCV by age 8.5 years (range 2.5–54 years) (Table 1).

By comparison, HCV RNA was detected by bDNA in all but one of the initial samples from eight controls with persistent HCV viraemia whose sera were stored under similar conditions and who were matched for age, sex, and interval from HCV exposure to phlebotomy (Fisher's exact $P = 0.0008$;

Table 2. Hepatitis C virus (HCV) RNA results of serial samples over the course of 10–20 years from 12 patients with viral clearance.

Subject	Interval from first exposure to first test	Initial HCV-positive test	Years tested for HCV RNA		Duration of HCV RNA persistence
			PCR-negative samples	PCR-positive samples	
1	4 years	1985	1985, 1986, 1999, 2001	None	Never detected
2	2 years	1980	1980, 1982, 1985, 1988, 1998, 1999	None	Never detected
3	2 years	1980	1980, 1982–1985, 1987, 1990, 1994, 1995, 2002	None	Never detected
4	2 years	1987	1987, 1999, 2000, 2002	None	Never detected‡
5	1.5 years	1986	1986–1988, 1996, 1997, 1999, 2001	None	Never detected
6	2 years	1985	1985–1988, 1995–2000, 2002	1991 (repeat negative)	Never detected
7	6 months	1986	1986, 1987, 1992, 1993, 1995, 2000	None	Never detected‡
8	4 years	1979	1979, 1982, 1991, 1993–1998, 2002	1981 (3720 IU/mL†)	1 year
9	7 months	1981*	1982, 1984, 1989, 1998, 2002	1981	3 months
10	2 years	1987*	1992, 1995, 1999, 2000, 2002	1987, 1989, 1991	5 years
11	6 months	1978*	1979, 1981, 1985, 1998, 1999, 2002	1978	1 year
12	2 months	1977*	1977–1980, 1983, 1984, 1988, 2001	1977	4 months‡

*Polymerase chain reaction (PCR)-positive. Others only anti-HCV positive.

†By branched-DNA (bDNA) quantitation.

‡Patients nos 4, 7 and 12 have been anti-HCV negative since 2002, 1992 and 1991, respectively.

Fig. 1. Hepatitis C virus (HCV) RNA results on initial anti-HCV positive samples from 12 patients with viral clearance (top panel) and eight controls with viral persistence (bottom panel). *Polymerase chain reaction (PCR)-negative, anti-HCV positive, grey bar – PCR-positive; branched-DNA (bDNA)-negative; bDNA cut-off = 615 IU/mL. Controls were matched for age, sex and duration of blood product exposure. Patients nos 1–3 and 11 lacked controls (see text).

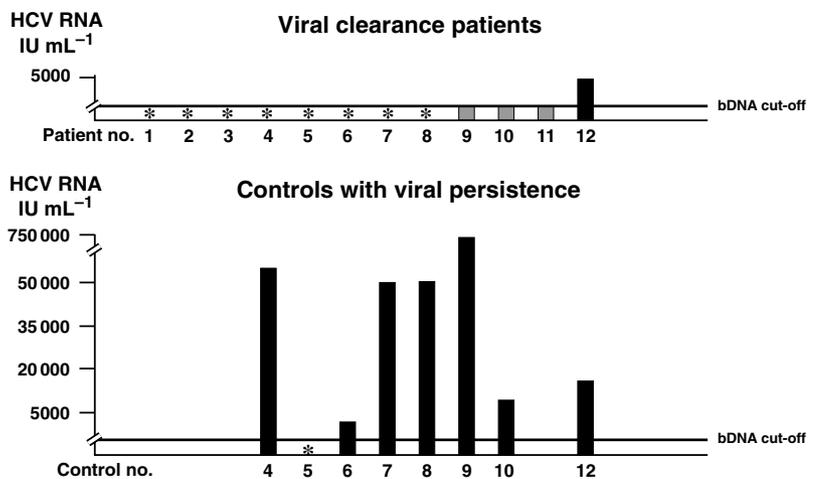


Fig. 1). Levels in these viraemic controls ranged from 4644 to 678 515 IU/mL (median 43 532 IU).

Polymerase chain reaction testing was performed on the clearance patients' samples that were collected longitudinally over the course of the next 10–20 years (median six samples per patient, range 3–10, Table 2). Of the four patients in whom HCV RNA was detected in initial samples, three (patients nos 9, 11 and 12) cleared the virus during the next year. HCV RNA persisted beyond 2 years in only one patient (patient 10) who had persistence of HCV RNA for 5 years before clearance, during which time he received only sterilized concentrates and had no intercurrent hepatitis A or B infections. All but

three of the 12 patients (nos 4, 7 and 12) retained anti-HCV reactivity by ELISA. These three patients seroreverted 6, 14 and 15 years respectively after HCV RNA was no longer detected in their sera.

In sequential testing, HCV RNA was detected in five of 44 samples collected when patients were receiving unsterilized concentrates or unsterilized plasma between 1977 and 1987 (Table 2). The positives included four initial samples (patients nos 9–12) and a 1981 sample with a low level (3720 IU/mL) from patient 8, who had been undetectable by PCR in 1979. In 53 samples collected after implementation of effective HCV sterilization and screening procedures, HCV RNA was reproducibly detected only in patient 10.

Patient 6 was presumed to have had a false positive test on a single sample drawn in 1991, 6 years after he had last received unsterilized blood products. That sample had been depleted, but another drawn a month earlier and seven drawn over the next 6 years were all negative.

Hepatitis C virus genotyping was successful in four patients, of whom three had genotype 1 (patients nos 8, 10 and 11) and one had genotype 2 (patient 9). None of the 12 clearance patients have had evidence of liver disease, and all 12 had persistently normal serum alanine aminotransferase (ALT) results.

Discussion

Previous studies of small numbers of patients with acute self-limited hepatitis have shown a median time to HCV RNA clearance of 12 weeks, with a range of 2–24 months [9,10,12,14]. Based on limited outcome measures such as these, it is felt that patients with spontaneous resolution of HCV infections almost always clear virus within the first 6 months to 1 year following acute infection, and that clearance is more likely in patients who are symptomatic during the primary infection.

Data on patients who develop HCV infections during long-term prospective studies are even more limited. In a prospective study of i.v. drug users followed at 6-month intervals [11], HCV seroconversion was recognized in 43 (30%) of participants who were followed a median of 72 months. Viral clearance was noted in six (14%). Five who were anti-HCV negative at study entry developed asymptomatic viraemia. Four of the five cleared virus in <2 years, while HCV RNA persisted for 42 months before spontaneous clearance in one patient. One patient developed anti-HCV without the detection of HCV RNA.

Our study of 12 patients with spontaneous HCV RNA clearance followed for 12–24 years following acute infection confirms that clearance usually occurs within the first 1–2 years after onset of infection. HCV RNA was undetectable in the initial samples of eight anti-HCV positive patients (66%). Three were positive by PCR only. In one, HCV RNA could be quantitated at very low levels with the bDNA assay. Maximum duration of infection before clearance was 7 months or less in four (36%) and 1–2 years in six (55%) of those who had yearly samples available for testing.

Limited clinical observations suggest that most cases of spontaneous resolution occur within the first 6 months to 1 year following acute infection [9–14]. Although our findings are in agreement with these

reports, one of our patients had persistence of HCV RNA for 5 years before clearance, during which time he received only sterilized concentrates. This finding demonstrates conclusively for the first time that viral clearance can occur in the absence of reinfection once a chronic HCV infection is established.

Our patients with viral clearance had either no detectable HCV RNA or low levels of viraemia very early in the course of the infection. In contrast, controls with persistent viraemia matched for age and duration of infection and followed for similar periods of time had significantly higher early levels of HCV RNA. Villano *et al.* [11] also found that peak HCV RNA levels were significantly lower among patients with viral clearance compared with patients with viral persistence. These findings suggest that those with lower viral loads following acute infection are more likely to clear their infections spontaneously.

At least half of our patients with viral clearance were re-exposed to unsterilized clotting factor concentrates prior to the widespread availability of virally inactivated clotting factor concentrates in the mid-1980s. However, only one showed possible evidence of reinfection and this persisted for <1 year. Fewer instances of viraemia in previously infected than in previously uninfected drug users with continuing drug use have been shown by Mehta *et al.* [19]. In most instances, reinfection was characterized by lower levels of viraemia.

We and others have previously shown that HCV clearance is associated with a younger age at infection and is more likely to occur in children than in adults receiving blood transfusions [5,18]. Other factors which have been shown to be related to clearance include black race and HIV infection [7], with the clearance rate being lower in African-Americans than in whites and in HIV-positive than in HIV-negative patients. All of our patients were white and HIV-negative.

Three of our 12 patients with spontaneous viral clearance subsequently lost antibody to HCV. Complete seroreversion following spontaneous clearance of HCV RNA appears to be very infrequent. In the Collaborative Transfusion Study from the National Heart, Lung and Blood Institute, six of 90 living individuals (7%) who acquired transfusion-associated hepatitis C in 1974 were negative for both HCV RNA and anti-HCV when studied 23 years later in 1997 [8].

The shortcomings of our study include the small sample size, the infrequent early sampling and the long duration of storage of early samples, which could have caused a loss in viral nucleic acid.

However, persistently viraemic controls infected for similar periods of 17–24 years had significantly higher initial levels of virus in serum specimens stored under similar conditions, arguing against loss of HCV RNA from prolonged storage.

It is uncertain whether patients with viral clearance have true resolution of their HCV infection. False negative PCR tests occur in a small number of patient. Furthermore, serum HCV RNA levels fluctuate and may be transiently undetectable in some individuals with chronic infections [11,20]. In such cases, it is possible that HCV RNA may be replicating in a hepatic reservoir, thus providing continued antigenic stimulation for the production of antibodies without the presence of viraemia. However, in a cohort of Irish women who were infected with HCV through anti-Rh immune globulin, HCV RNA could not be identified in liver biopsy tissue from those in whom HCV RNA was undetectable in serum by PCR [21]. Likewise, in two large multicentre studies, HCV RNA could not be detected in the liver of patients with sustained viral remissions following treatment with α -interferon [22,23], supporting the belief that a negative serum PCR reflects cleared past-exposure in liver tissue.

It is difficult to know from a single negative HCV RNA determination whether the acute infection has resolved or whether the individual has a 'stuttering' chronic infection. Our data provide strong evidence for the natural resolution of infection in untreated anti-HCV positive haemophiliacs who have persistently normal ALT results and no detectable HCV RNA with sensitive PCR tests on two consecutive occasions over a period of a year or more.

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