MAJOR ARTICLE

Prospective Follow-Up of Patients with Acute Hepatitis C Virus Infection in Brazil

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Background. The natural outcome of infection with hepatitis C virus (HCV) varies substantially among individuals. However, little is known about host and viral factors associated with a self-limiting or chronic evolution of HCV infection.

Methods. From 1 January 2001 through 31 December 2008, a consecutive series of 65 patients from Rio de Janeiro, Brazil, with a well-documented diagnosis of acute HCV infection, acquired via various routes, were enrolled in this study. Patients were prospectively followed up for a median of 40 months after the estimated date of HCV infection with serial measurements of serum alanine aminotransferase, HCV RNA, and anti-HCV antibodies. Spontaneous viral clearance (SVC) was defined as undetectable levels of HCV RNA in serum, in the absence of treatment, for 3 consecutive HCV polymerase chain reaction tests within the first 6 months of follow-up. Cox proportional hazards regression was used to identify host and viral predictors of SVC.

Results. The cumulative rate of SVC was 44.6% (95% confidence interval, 32.3%–57.5%). Compared with chronic HCV evolution, patients with self-limiting disease had significantly lower peak levels of anti-HCV antibodies (median, 109.0 vs 86.7 optical density–to–cutoff ratio [od/co]; $P < .02$), experienced disease symptoms more frequently (69.4% vs 100%; $P < .001$), and had lower viral load at first clinical presentation (median, 4.3 vs 0.0 log copies; $P = .01$). In multivariate analyses, low peak anti-HCV level (<93.5 od/co) was the only independent predictor for SVC; the hazard ratio compared with high anti-HCV levels (⩾93.5 od/co) was 2.62 (95% confidence interval, 1.11–6.19; $P = .03$).

Conclusion. Our data suggest that low levels of anti-HCV antibodies during the acute phase of HCV infection are independently related to spontaneous viral clearance.

Although hepatitis C virus (HCV) accounts for only a small proportion of cases of clinical acute hepatitis, it is a major cause of chronic liver disease and hepatocellular carcinoma in both developed and developing countries [1–3]. The global prevalence of HCV was estimated at 3%, with a total of 170 million persons infected worldwide; in the United States, nearly 2% of the population is infected [4–6].

HCV infection may be self-limiting and can spontaneously resolve before proceeding beyond the acute phase or may persist, leading to chronic infection [1–3]. Reported rates of spontaneous HCV resolution from longitudinal studies substantially vary, with estimates ranging from 10% to 60% [4, 7–13]. Approximately 80% of patients with self-limiting hepatitis experience HCV RNA clearance within 3 months of disease onset [14–16]. Persistent viremia beyond 6 months of infection is usually associated with chronic evolution [1, 7, 9, 17]. The mechanisms responsible for the relatively

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Table 1. Individual Patient Characteristics

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high rate of chronicity in HCV infection are still poorly understood, although it has been speculated that disease outcome is determined by a complex virus-host interplay in the early phase of infection [18, 19].

Several host and viral factors, including type of exposure, HCV viral load, HCV genotype, sex, ethnicity, age, occurrence of disease symptoms, polymorphisms in the *IL28B* gene, and specific HLA alleles, have been associated with spontaneous viral clearance (SVC) [1–3, 11, 20–23]. However, given (1) widely heterogeneous study populations in previous investigations, (2) small sample sizes due to common difficulties in diagnosis of acute HCV infection, and (3) unstandardized definition of both acute HCV infection and SVC [24], conclusive epidemiologic data on predictors for SVC in acute HCV infection remain sparse.

We present epidemiologic data and clinical characteristics of a cohort of 65 consecutive individuals with a well-defined diagnosis of acute HCV, acquired via various routes, prospectively followed up from the initial phase of disease in Rio de Janeiro, Brazil, from 1 January 2001 through 31 December 2008. We aimed to investigate the rate of SVC and to identify host and viral factors to predict a self-limiting or chronic evolution of HCV infection.

METHODS

Patients and definitions. In January 2001, the Viral Hepatitis Clinic at the Oswaldo Cruz Institute, FIOCRUZ, together with the Central Public Health Laboratory Noel Nutels, Rio de Janeiro, Brazil, initiated a screening program for the early diagnosis of acute viral hepatitis. Patients referred to the clinic were either symptomatic (ie, jaundice and/or dark urine) with elevated alanine aminotransferase (ALT) levels or were asymptomatic with recent anti-HCV seroconversion. The latter consisted of regular blood donors or individuals with recent unintentional exposure to HCV-infected biological material. Among those who were symptomatic, initial visits included medical evaluation and testing for serologic markers for viral hepatitis A, B, and C and leptospirosis along with ALT. Individuals with elevated ALT levels but no positive serologic test results were tested for hepatitis A virus RNA, hepatitis B virus DNA, and HCV RNA and underwent follow-up tests for all serologic markers to exclude the possibility that they presented during the window period between onset of viremia and seroconversion. Further testing for antibodies (IgM and IgG) against other hepatotropic viruses (cytomegalovirus, herpes simplex virus types 1 and 2, Epstein-Barr virus, dengue, and hepatitis E virus) was performed. Abdominal ultrasonography was conducted in all patients as a complementary diagnostic tool for possible advanced cases of chronic liver diseases, such as cirrhosis and portal hypertension.

Diagnosis of acute or early HCV infection was based on the following established criteria [23–25]: (1) a positive anti-HCV antibody test result or HCV RNA polymerase chain reaction (PCR) assay result in a participant with a documented negative anti-HCV test result within the past year or (2) a positive anti-HCV assay result in a participant with clinical hepatitis, detectable serum HCV RNA, a serum ALT level 10 times the upper limit of normal (32 U/L), and negative results of tests for hepatitis B surface antigen and hepatitis A IgM antibody or, in the absence of detectable HCV RNA, a history of high-risk exposure between 1 and 3 months before clinical manifestation in HCV-seropositive patients. High-risk exposure was defined as medical (surgical interventions, any endoscopic procedures, being in health units with intravenous access) or dental procedures, paraphernalia sharing among drug users, tattooing, and piercing.

The date of HCV infection was estimated as the day of high-risk exposure if available or, in the absence of this information, as either 6 weeks before the onset of symptoms in symptomatic patients [16, 23] or 6 weeks before seroconversion in asymptomatic patients [23, 26]. Seroconversion was defined as a positive anti-HCV antibody test result or HCV RNA PCR assay result in a participant with a documented negative result of an anti-HCV test within the past year. The date of seroconversion was defined as the midpoint between the last anti-HCV negative test result and the first anti-HCV positive test result.

Diagnoses were made by trained experienced physicians after evaluation of the patient's clinical presentation and laboratory data. Patients were followed up prospectively with scheduled visits for clinical and laboratory evaluation weekly for the first month of prospective follow-up, every 2 weeks for the second and third months, monthly until 12 months, 4 times a year for the second and third year, 3 times a year for the fourth year, and at least once a year thereafter. Serial measurements of serum ALT, HCV RNA, and anti-HCV antibodies were performed. Epidemiologic data concerning risk factors were obtained during medical evaluation, and HCV testing was extended to household members or sexual partners. Serial blood samples were drawn at each visit for biochemical and virologic evaluation and to evaluate cellular and humoral immune responses. Patients who did not clear HCV RNA before the fourth month of follow-up were assigned to the public hospital for eventual antiviral therapy. Treatment protocols varied according to medication availability and clinician's judgment. None of the study participants started antiviral treatment within the first 6 months after the estimated date of HCV infection.

SVC was defined as undetectable HCV RNA level within the first 6 months of follow-up after the estimated date of infection

Table 2. Characteristics of the Study Population

Characteristics	Patients (n = 65)
Total no. of HCV cohort, 2001–2008	73
Eligible patients for analysis[a]	65
Follow-up, mean ± SD (median, range), months	47.6 ± 28.6 (40.4, 7–108)
Age, mean ± SD (range), years	45.7 ± 12.4 (20–77)
Female	40 (61.5)
Race/ethnicity	
White	23 (35.4)
Mixed	34 (52.3)
Black	6 (9.2)
Other	2 (3.1)
Risk factor or mode of infection	
Medical procedure[b]	32 (49.2)
Unprotected sex	6 (9.2)
HCV-positive partner	14 (21.5)
Hemodialysis	8 (12.3)
Other[c]	5 (7.7)
HCV genotype	
Type 1	50 (76.9)
Type 2	4 (6.2)
Type 3	9 (13.8)
Type 4	1 (1.5)
Disease symptoms[d]	54 (83.1)
Time to disease symptoms,[e] mean ± SD (range), days	50.8 ± 19.7 (19–129)
Seroconversion[f]	26 (40.0)
Peak ALT level during acute phase,[g] mean ± SD (median, range), U/L	1308 ± 874 (1110, 65–3985)
Peak level of anti-HCV antibodies during acute phase,[h] mean ± SD (median, range), od/co	97.8 ± 35.2 (93.5, 0.5–172.2)
Viral load at first visit (median, range), copies/mL	8897 (0–400, 448–700)
Antiviral treatment during acute phase (≤6 months from infection)	0 (0.0)
Antiviral treatment during late follow-up (>6 months from infection)	17 (25.0)
Start treatment after infection, mean ± SD (median), months	17.5 ± 9.8 (13.1)

NOTE. Data are no. (%) of patients, unless otherwise indicated. ALT, alanine aminotransferase; HCV, hepatitis C virus; od/co, optical density–to–cutoff ratio; SD, standard deviation.

[a] Patients with human immunodeficiency virus coinfection (n = 4), hepatitis B virus coinfection (n = 1), heavy drinking (n = 1) pregnancy (n = 1), and/or hepatotoxic medication use (n = 1) were excluded.

[b] Includes minor or major surgery, hospitalization, and/or blood transfusion.

[c] Includes sharing of sharp personal items (n = 3) and blood exposure (n = 2).

[d] Including jaundice and/or dark urine.

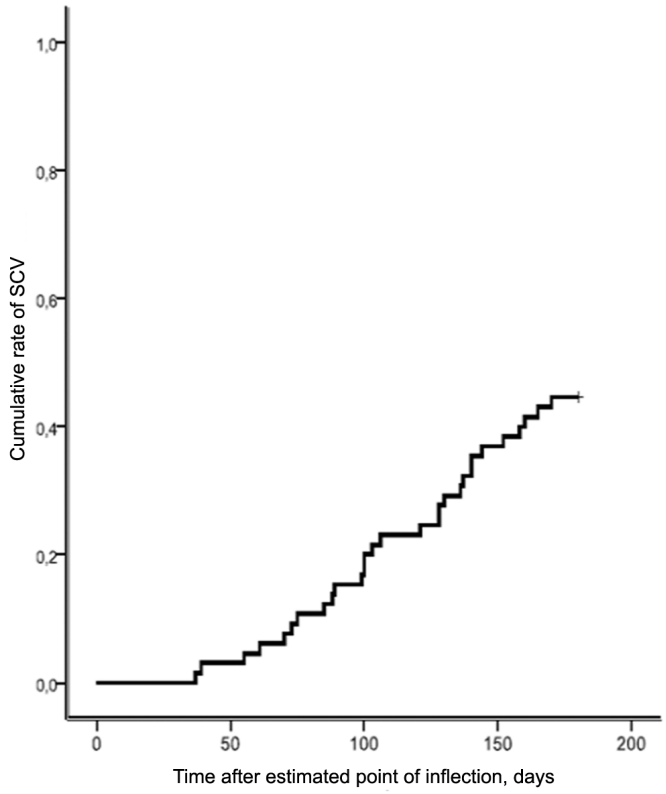
[e] Calculated from the estimated date of infection.

[f] Defined as a positive HCV antibody test or HCV RNA polymerase chain reaction assay result in a participant with a documented negative result on an anti-HCV test within the past year.

[g] Within the first 6 months after the estimated date of infection.

[h] Within the first 6 months after the estimated date of infection. Note that for hemodialysis patients (n = 8), because of a delayed immune response, the first 8 months of follow-up were considered for the peak anti-HCV measure.

with qualitative Cobas Amplicor molecular assays (lower detection limit, 50 IU/mL) performed in serum in the absence of treatment. To sustain SVC classification, at least 2 additional consecutive test results indicating undetectable HCV RNA levels were required because intermittent viremia has frequently been observed in the early phase of HCV infection [12, 27]. The date of SVC was defined as the midpoint between the date of the first of 3 consecutive samples with undetectable HCV RNA levels and the date of the last sample with detectable HCV RNA levels. In the event that the first sample collected had undetectable HCV RNA levels, the date of SVC was estimated as the midpoint between the date of infection and the date of the first sample with undetectable HCV RNA levels [23, 25]. This study was approved by all participating institutional review boards, and signed informed consent was obtained from all participants.



Days	50	100	150	180
No. at risk	63	54	41	36
Cumulative SVC, No. (%) (95% CI)	2/65 (3.1) (0.4-10.7)	11/65 (16.9) (8.8-28.3)	24/65 (36.9) (25.3-49.8)	29/65 (44.6) (32.3-57.5)

Figure 1. Kaplan-Meier failure estimates of time to spontaneous viral clearance (SVC) in 65 patients with acute hepatitis C virus, Rio de Janeiro, Brazil, 2001–2008. CI, confidence interval.

Serum HCV RNA detection, quantification, genotyping, and serotyping. Serum samples were obtained from whole blood collected in tubes without anticoagulants and separated within 4 h of venipuncture, aliquoted, and stored at −80°C, except for 1 vial that was used for determining ALT levels. Serum samples were tested for anti-HCV by the AxSYM HCV 3.0 microparticle enzyme immunoassay (Abbott Diagnostics). The qualitative determination of HCV RNA was performed by the Cobas Amplicor HCV test (detection limit, 50 IU/mL) and the quantitative determination through real-time PCR assay [28] (detection limit, 1000 copies/mL). Genotype was determined by using the INNO-Lipa HCV II assay (Bayer Diagnostics) or by HCV serotyping in patients with low or undetectable viral loads. HCV serotypes were determined using the Murex HCV Serotyping 1–6 assay (Abbott) [29]. To investigate possible transmission routes, we submitted suspected source and case serum samples to direct nucleotide sequencing of the NS5B region and analyzed them on an automatic sequencer (ABI Prism 3100 Genetic Analyser; Applied Biosystems).

Statistical analysis. Kaplan-Meier curves were used to estimate cumulative rates of SVC at 6 months after the estimated date of HCV acquisition. Univariate comparisons between patients with self-limiting and chronic HCV infection were performed using the 2-tailed independent-samples *t* test for continuous variables and the χ^2 test and the Fisher exact test for categorical variables. To test for normal distribution of continuous parameters, we used the Kolmogorov-Smirnov test. For skewed variables, logarithmic transformation was applied.

Cox proportional hazards regression was used to estimate multivariate hazard ratios and their 95% confidence intervals (CIs) for the association of SVC with host and viral factors. Follow-up started at the estimated date of HCV infection and ended at the estimated date of SVC or at censoring (at day 180 after the estimated date of infection). Sensitivity analyses were performed by estimating alternate HCV acquisition dates using (1) 60 days before symptom onset for symptomatic patients and (2) 6 weeks before the first positive antibody test result or 1 week before the first positive HCV RNA test result for asymp-

Table 3. Comparison of Baseline Demographic, Clinical, and Virologic Characteristics of Patients with Self-Limiting and Chronic Hepatitis C Virus (HCV) Courses, Rio de Janeiro, Brazil, 2001–2008

Characteristic	Patients with self-limiting infection ($n = 29$)	Patients with chronic infection ($n = 36$)	P
Age, mean ± SD (range), years	44.1 ± 11.4 (25–73)	47.0 ± 13.2 (20–77)	.24
Female	19 (65.5)	21 (58.3)	.61
Disease symptoms[a]	29 (100.0)	25 (69.4)	<.001
Time to symptoms, mean ± SD (range), days	48.3 ± 15.5 (19–89)	53.7 ± 23.8 (24–129)	.75
Race/ethnicity			
White	10 (34.5)	13 (36.1)	.51
Mixed	17 (58.6)	17 (47.2)	
Black	2 (6.9)	4 (11.1)	
Other	0 (0.0)	2 (5.6)	
Risk factor or mode of infection			
Parenteral[b]	18 (62.1)	27 (75.0)	.29
Sexual[c]	11 (37.9)	9 (25.0)	
HCV genotype			
Type 1	22 (75.9)	28 (77.8)	.18
Type 2	0 (0.0)	4 (11.1)	
Type 3	5 (17.2)	4 (11.1)	
Type 4	1 (3.4)	0 (0.0)	
Seroconversion[d]	8 (27.6)	18 (50.0)	.08
Peak ALT level during acute phase,[e] mean ± SD (median), U/L	1397 ± 800 (1224)	1236 ± 937 (1008)	.47
Peak level of anti-HCV antibodies during acute phase,[f] mean ± SD (median), od/co	85.7 ± 33.3 (86.7)	107.6 ± 34.1 (109.0)	.015
Log viral load at first visit, mean ± SD (median), copies/mL	2.05 ± 2.57 (0.0)	4.01 ± 2.6 (4.3)	<.01

NOTE. Data are no. (%) of patients, unless otherwise indicated. Self-limiting infection was defined as a series of at least 3 negative HCV RNA test results within 6 months after the estimated point of infection. Patients with detectable HCV RNA beyond 6 months after the estimated point of HCV infection were classified as having chronic disease. ALT, alanine aminotransferase; od/co, optical density–to–cutoff ratio; SD, standard deviation.

[a] Including jaundice and/or dark urine.
[b] Includes minor or major surgery, hospitalization, blood transfusion, blood exposure, and/or hemodialysis.
[c] Includes unprotected sex and/or HCV-positive partner.
[d] Defined as a positive HCV antibody test or HCV RNA polymerase chain reaction assay result in a participant with a documented negative result on an anti-HCV test within the past year.
[e] Within the first 6 months after the estimated point of infection.
[f] Within the first 6 months after the estimated point of infection. Note that for hemodialysis patients ($n = 8$), because of a delayed immune response, the first 8 months of follow-up were considered for the peak anti-HCV measure.

tomatic patients [23]. The proportional hazards assumption was checked using Schoenfeld residuals and visual inspection of the hazard plots. For metric data (including ALT, HCV RNA, and anti-HCV antibodies), we first computed adjusted hazard ratios with 95% CIs using the median value of the distribution as the cutoff point; in sensitivity analyses, we recalculated all results, retaining all metric data in continuous form. Two-sided $P < .05$ was considered statistically significant. All statistical analyses were conducted using SPSS statistical software, version 15.0 (SPSS) statistical software.

RESULTS

Patient characteristics. Individual patient characteristics detailing the course of 65 consecutive patients with acute HCV infections in Rio de Janeiro, Brazil, diagnosed from 2001 through 2008 are listed in Table 1. An additional 8 patients were excluded from the present analysis because they had been identified with human immunodeficiency virus coinfection ($n = 4$), hepatitis B virus coinfection ($n = 1$), heavy drinking ($n = 1$), pregnancy ($n = 1$), and/or hepatotoxic medication use ($n = 1$).

Median follow-up after the estimated point of HCV acquisition to the last available HCV RNA measurement was 40.4 months (Table 2). Forty women (61.5%) and 25 men (38.5%) comprised the study cohort. The mean age at HCV infection was 45.7 years (range, 20–77 years). The major risk factor or source of HCV infection was undergoing medical procedures (including hospitalization, minor or major surgery, and/or blood transfusion) (49.2%), providing a precise putative date of infection. Other sources of HCV infection were having an HCV-positive partner (21.5%) or undergoing hemodialysis

Table 4. Demographic, Clinical, and Virologic Factors Associated with Spontaneous Viral Clearance (SVC) in 65 Patients with Acute Hepatitis C Virus (HCV), Rio de Janeiro, Brazil, 2001–2008

Characteristic[a]	Proportion (%) of patients who experienced SVC[b]	Multivariate hazard ratio for SVC (95% CI)[c]	*P*
Age			
⩾44 years	11/32 (34.4)	1.00	
<44 years	18/33 (54.5)	1.52 (0.61–3.77)	.37
Sex			
Male	10/25 (40.0)	1.00	
Female	19/40 (47.5)	1.35 (0.57–3.21)	.50
Mode of infection			
Parenteral	18/45 (40.0)	1.00	
Sexual	11/20 (55.0)	1.05 (0.43–2.56)	.92
Seroconversion[d]			
Yes	8/26 (30.8)	1.00	
No	21/39 (53.8)	1.96 (0.65–5.92)	.23
Peak ALT level[e]			
<1110.5 U/L	14/32 (43.8)	1.00	
⩾1110.5 U/L	15/31 (48.4)	1.03 (0.43–2.50)	.95
Peak level of anti-HCV antibodies[f]			
⩾93.5 od/co	9/30 (30.0)	1.00	
<93.5 od/co	18/30 (60.0)	2.62 (1.11–6.19)	.028
Log viral load first visit			
⩾3.95 copies/mL	10/32 (31.3)	1.00	
<3.95 copies/mL	19/33 (57.6)	1.33 (0.56–3.18)	.52

NOTE. ALT, alanine aminotransferase; CI, confidence interval; od/co, optical density–to–cutoff ratio.

[a] For continuous covariables, the median value of the distribution was used as the cutoff.

[b] SVC was defined as a series of at least 3 negative HCV RNA results within 6 months after the estimated point of infection. Patients with detectable HCV RNA beyond 6 months after the estimated point of HCV infection were classified as having chronic disease.

[c] Estimated from Cox proportional hazards regression analyses adjusted for all patients characteristics.

[d] Defined as a positive HCV antibody test or HCV RNA polymerase chain reaction assay result in a participant with a documented negative result on an anti-HCV test within the past year.

[e] Within the first 6 months after the estimated date of infection.

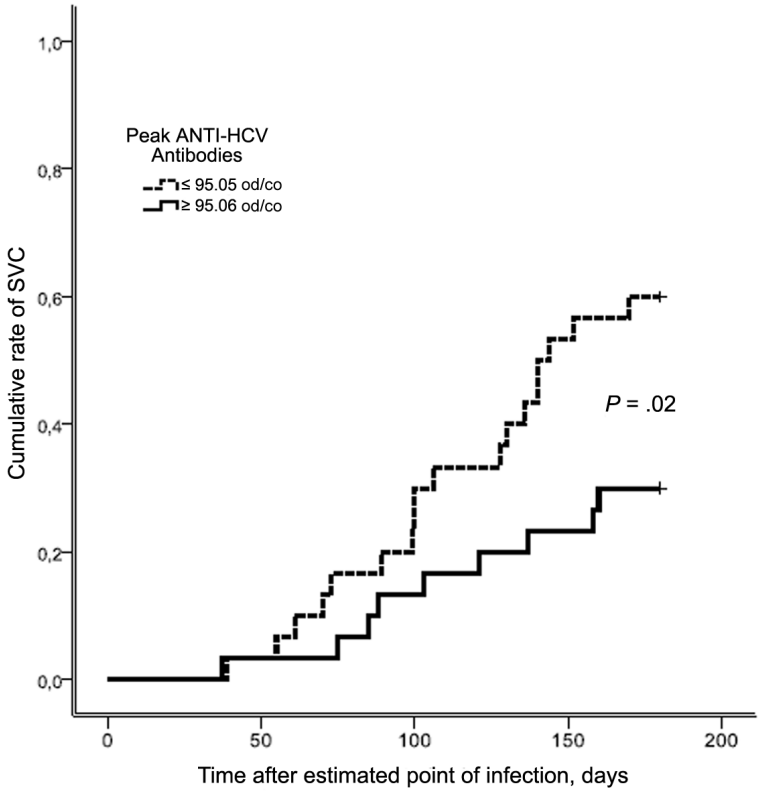
[f] Within the first 6 months after the estimated date of infection. Note that for hemodialysis patients ($n = 8$), because of a delayed immune response, the first 8 months of follow-up were considered for the peak anti-HCV measure.

(12.3%). During the first 6 months of follow-up, 54 (83.1%) of the 65 patients experienced disease symptoms (including jaundice and/or dark urine); the mean time from the estimated date of HCV infection to onset of disease symptoms was 50.8 days (range, 19–129 days). Genotype 1 was predominant among the group (76.9%), followed by genotype 3 (13.8%) and genotype 2 (6.2%). Median viral load at first clinical presentation was 8897 copies/mL (range $\log_{10}$, 0–8.60 copies/mL). Peak ALT and peak anti-HCV antibody levels during the first 6 months of follow-up ranged from 65 to 3985 U/L and from 0.5 to 172 optical density–to–cutoff ratio [od/co], respectively (Table 2).

Spontaneous HCV clearance. The cumulative rate of SVC within the first 6 months of follow-up was 44.6% (95% CI, 32.3%–57.5%; Figure 1). The median time to SVC was 106 days (range, 37–170 days). An additional 7 patients (10.8%) with persistent or intermittent viremia during the first 6 months of follow-up cleared the virus during later follow-up, of whom 5 cleared from 6 to 12 months and 2 from 2 to 3 years (Table 1). However, those patients were not classified as spontaneous HCV clearers but censored at day 180 in our statistical models.

Predictors of spontaneous HCV clearance. Patients with a self-limiting course of HCV infection experienced disease symptoms more frequently (100% vs 69.4%; $P < .001$), had lower peak levels of anti-HCV antibodies during the first 6 months of follow-up (median, 86.7 vs 109.0 od/co; $P = .015$), and had lower viral loads at first clinical presentation (median, 0.0 vs 4.3 $\log_{10}$ copies; $P = .01$). No statistically significant differences were found in other host and/or viral factors, including age ($P = .24$), sex ($P = .61$), risk factors ($P = .29$), seroconversion ($P = .08$), peak ALT level ($P = .47$), and HCV virus genotype ($P = .18$) between the groups (Table 3).

In multivariate regression analysis, including all the patient



Group/Days	50	100	150	180
≤95.05 od/co	1/30 (3.3)	9/30 (30.0)	15/30 (50.0)	18/30 (60.0)
>95.05 od/co	1/30 (3.3)	4/30 (13.3)	7/30 (23.3)	9/30 (30.0)

Figure 2. Kaplan-Meier failure estimates of spontaneous viral clearance (SVC) according to peak anti–hepatitis C virus (HCV) antibodies within the first 6 months of follow-up in 65 patients, Rio de Janeiro, Brazil, 2001–2008. Note that the median value of the distribution (95.05 optical density–to–cutoff ratio [od/co]) was used as the cutoff.

characteristics as covariates, only peak anti-HCV level during the first 6 months of follow-up was independently related to SVC; the hazard ratio for low level of anti-HCV antibodies (<93.5 od/co) compared with high level of anti-HCV antibodies (⩾93.5 od/co) was 2.62 (95% CI, 1.11–6.19; $P = .028$; Table 4 and Figure 2). Exclusion of patients undergoing hemodialysis did not change our findings in any notable way (Table 5).

When using alternate HCV acquisition dates in sensitivity analysis, simultaneously modeling log viral load, peak ALT level, and peak anti-HCV antibody level as continuous variables in our regression runs, peak level of anti-HCV antibodies during the first 6 month of follow-up remained an independent predictor for SVC; the hazard ratio per anti-HCV antibody unit increase was 0.98 (95% CI, 0.97–0.99; $P = .03$; Table 6).

DISCUSSION

Our study presents epidemiologic data from a series of 65 individuals with a well-documented diagnosis of acute HCV infection who were prospectively followed up from the initial phase of disease in Rio de Janeiro, Brazil. To our knowledge, this is the largest such set of patients described from South America.

Our data confirm spontaneous viral resolution to occur in a considerable percentage of patients. Similar to our estimate of 44.6%, Sharaf Eldin and coworkers [25], from a symptomatic cohort of patients with acute HCV in Egypt, recently reported a rate of SVC at 6 months equaling 41.5%. Notably, the clearance rate of 44.6% reported in the present study may be an underestimate because spontaneous HCV resolution may extend beyond the 6-month period, as previously shown [11] and also observed in our population. Santantonio and colleagues [18] from an Italian cohort of 40 patients with community-acquired acute HCV infection reported a rate of SVC of 30%. In contrast, Wang and coworkers [23] and Page and coworkers [30], from 2 distinct US cohorts, reported rates of SVC of 18% and 20%, respectively. However, in those cohorts most partic-

Table 5. Demographic, Clinical, and Virologic Factors Associated with Spontaneous Viral Clearance (SVC), after Excluding Hemodialysis Patients, Rio de Janeiro, Brazil, 2001–2008

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

ipants were asymptomatic and/or injection drug users, possibly explaining the lower rates of SVC compared with the findings of the present study of predominantly symptomatic females. Both female sex and disease symptoms have been shown to be associated with favorable outcome in acute HCV infection [11].

There are some specifics of our cohort that are noteworthy. First, because patients were referred to our clinic on the account of a suspicion of possible acute hepatitis, they might not represent the overall spectrum of disease that is mostly asymptomatic; however, the inclusion of both symptomatic and asymptomatic patients minimizes the likelihood of possible referral bias. Second, more than 20% of patients had sexual partners who were identified as having HCV infection during the study period. HCV RNA was detected in 13 of 14 sexual partners, all of whom shared the same HCV genotype. The only sexual partner who had undetectable HCV RNA was also in the acute phase of HCV infection and had cleared the virus spontaneously.

In univariate analysis, we observed that symptomatic infection and low viral load at first clinical presentation were both associated with higher rates of SVC, whereas we found no association for age, sex, race, peak ALT level, mode of HCV transmission, and/or HCV genotype. Similarly, in the Italian cohort [18], older age and jaundice were predictive of resolution, whereas there was no correlation with other host or viral factors. In a series of 12 patients with acute HCV infection, Hofer and colleagues [16] previously observed lower baseline viral loads in those in whom the virus spontaneously cleared compared with chronically infected persons (171,451 ± 66,421 IU/mL vs 396,759 ± 227,311 IU/mL); however, likely because of poor statistical power, this difference did not reach statistical significance. The authors further report that serial ALT measurements failed to discriminate patients with SVC from those developing chronic infection [16].

In our multivariate regression runs, low peak level of anti-HCV antibodies during the first 6 months of follow-up was the only significant predictor for SVC, persisting after exclusion of hemodialysis patients and under different modeling strategies. To date, there is little information on the timing, magnitude, specificity, and clinical relevance of the antibody response to acute HCV infection [26]. Unlike other viral infections in which the humoral response has a major role in viral clearance, antibodies are relatively delayed in HCV and ineffective because of rapid mutation in targeted antibody epitopes [31]. Even though the humoral immune response seems insufficient for viral clearance or protection against additional infection, it might play a role in containing viral replication and modulates chronic disease [32]. Descriptive trends for humoral immunity have been observed previously, suggesting decreases or even loss of antibodies over time in recovered chimpanzees and human subjects, different from what is observed in long-term carriers, who seem to maintain or even increase their levels of circulating HCV antibodies [26, 33–35]. Consistently, Huang and colleagues [36] in a cross-sectional study reported an inverse correlation between the signal-cutoff antibody ratio with HCV viremia, and Lu et al [37] described persistently low signal-cutoff ratios in 18 patients with spontaneous viral resolution, suggesting that signal-cutoff ratios might not increase significantly in those who spontaneously recover. The absence or loss of HCV antibody in patients who have cleared the virus is most likely due to the lack of antigenic stimulation to induce detectable antibody production after resolution of infection. Studies in chimpanzees have already shown that low-dose inoculums may be ineffective in inducing detectable HCV antibodies and once challenged with higher doses will seroconvert, but antibodies are short-lived in the absence of viremia [38].

This study has some potential limitations. First, the sample size, although relatively large compared with other investigations, was insufficient to obtain precise estimates under some of our analytic strategies, including the examination of possible interaction among the single predictors. Second, because of the absence of asymptomatic patients in the group with self-limiting HCV infection, we were unable to estimate multivariate risk ratios for the effect of disease symptoms on SVC. Finally, in some patients the date of HCV infection had to be estimated on the basis of established criteria because the exact date of high-risk exposure was not available. However, sensitivity analysis using alternate infection dates yielded similar estimates, suggesting that the impact of this limitation is negligible.

In summary, data from the present longitudinal study confirm that spontaneous viral resolution occurs in a relatively large proportion of patients with symptomatic acute HCV infection. Different from previous studies that have already qualitatively described profiles of humoral immune response during the acute phase of infection, we here identified low antibody values as a statistically significant predictor for early viral clearance. Although our findings need to be confirmed in other populations, they underline that time for SVC should be con-

Table 6. Factors Associated with Spontaneous Viral Clearance (SVC) in 65 Patients with Acute Hepatitis C Virus (HCV) Infection, Using Alternate HCV Acquisition Dates, Rio de Janeiro, Brazil, 2001–2008

This table is available in its entirety in the online version of *Clinical Infectious Diseases*

sidered before antiviral treatment is initiated, particularly in symptomatic patients with low anti-HCV antibody levels in the early phase of disease.

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