

Hepatitis C Virus Kinetics During and Immediately After Liver Transplantation

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The study of hepatitis C virus (HCV) kinetics after liver transplantation (LT) might be important to design strategies to prevent HCV infection of the graft. We analyzed HCV kinetics during and immediately after LT in 20 consecutive patients undergoing LT for HCV-related cirrhosis. HCV RNA was quantified in blood samples obtained at regular intervals before, during, and after transplantation. HCV-RNA concentrations decreased in 18 of 20 patients during the anhepatic phase (mean decay slope -0.92 , mean HCV elimination half-life 2.2 hours). We found a significant correlation between the HCV viral load decay and the blood loss during the anhepatic phase, indicating that the observed HCV clearance rates are maximum estimates. In fact, in 1 patient with an unusually long anhepatic phase of 20 hours and with minimum blood loss, the HCV elimination half-life was 10.3 hours. Eight to 24 hours after graft reperfusion a sharp decrease in HCV viral load occurred in 19 patients (mean decay slope -0.34 , mean HCV elimination half-life 3.44 hours). HCV RNA became undetectable in only 1 patient. During the following days, HCV-RNA concentrations increased rapidly in 10 patients (mean HCV doubling time 13.8 hours), remained at similar levels in 4, and continued to decrease in 6. The only variable associated with a second-phase viral load decay was the absence of corticosteroids as part of the immunosuppressive regimen. In conclusion, a sharp decrease in HCV viral load occurs during the anhepatic phase and immediately after graft reperfusion, most likely owing to a lack of virion production and hepatic viral clearance. HCV infection of the graft, however, is an extremely dynamic process and viral replication begins a few hours after LT. (HEPATOLOGY 2002;35: 680-687.)

Cirrhosis caused by hepatitis C virus (HCV) infection is the main indication of liver transplantation (LT) in most transplant programs. Infection of the liver graft after transplantation is almost universal and persistent infection leading to chronic hepatitis, cirrhosis, and graft failure is common.^{1,2} In our geographic area, 30% of patients undergoing LT for HCV-related liver disease are already cirrhotic 5 years after transplantation.³ Regrettably, prophylaxis of HCV infection of the graft is not feasible because no specific anti-HCV immune globulin is available. In addition, antiviral treatment in patients on waiting lists for LT appears to be poorly effective and may cause severe adverse ef-

fects.^{4,5} Currently, treatment of hepatitis C infection after LT seems the most feasible strategy to eradicate HCV.⁶⁻⁸ Treatment of HCV infection after LT can be initiated before liver damage occurs or once liver disease is already established. Treatment in the early phase of LT seems a reasonable approach because eradication of HCV would prevent liver damage.⁹⁻¹¹ However, there are only a few studies analyzing the efficacy of antiviral therapy in the early posttransplantation period. Interferon monotherapy is not effective in achieving sustained virologic response in this setting, though histologic disease recurrence seems to occur less frequently¹⁰ and the appearance of biochemical hepatitis is delayed.¹¹ Combination therapy using interferon and ribavirin started as soon as 3 weeks after transplantation appears to be more effective than interferon monotherapy,⁹ but controlled studies are necessary to confirm its efficacy.

HCV viral kinetics after LT was not considered to design the therapeutic regimens used in any of the previously mentioned studies.⁹⁻¹¹ Information on HCV kinetics after transplantation is limited, but the data available indicate that HCV RNA decreases dramatically (even disappears) in most patients after transplantation and increases a few days after graft reperfusion.¹² Knowledge of viral kinetics has led to a better understanding of the mechanisms of drug action, emergence of resistant strains, and development of models to predict the time needed to eradicate all viral reservoirs.¹³ In HCV-infected immunocompetent patients the efficacy of antiviral therapy is strongly influenced by virologic vari-

Abbreviations: HCV, hepatitis C virus; LT, liver transplantation; RT-PCR, reverse transcription-polymerase chain reaction; CI, confidence interval.

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ables such as the HCV-infecting genotype and viral load.^{14,15} In the near future, more sophisticated data, such as viral kinetics in the early phase of antiviral therapy, will be used to predict treatment efficacy and to decide therapeutic regimens.^{16,17}

We have studied HCV kinetics during and immediately after LT in 20 patients undergoing LT for HCV-related cirrhosis. A careful analysis of the changes in HCV-RNA concentration during and after LT has revealed the existence of different phases of HCV kinetics that might facilitate the design of new strategies to prevent HCV recurrence after LT.

Patients and Methods

Patients. Twenty consecutive patients undergoing LT for HCV-related cirrhosis from September 2000 to March 2001 were included in the study. The indication for transplantation was decompensated cirrhosis in 9 patients and hepatocellular carcinoma in the remaining 11 patients. Four of the 20 patients received the right hepatic lobe of a living donor. All patients were HCV-RNA positive in at least 1 determination performed while on waiting list for LT. Serum samples were taken immediately before liver transplantation, at the beginning, and at the end of the anhepatic phase, and at 4, 8, 12, 16, 24, 48, 72, 96, and 120 hours after graft reperfusion. Thereafter, samples were obtained weekly during the first month and at weeks 12 and 24. During the surgical procedure, blood loss and transfusion requirements were recorded for each of the different phases (hepatectomy, anhepatic phase, and reperfusion phase). In hemodynamically stable patients, the Swan-Ganz catheter (Baxter, Irvine, CA), which is usually left the first 48 hours after transplantation, was replaced by a catheter in the hepatic veins. The catheter was placed under radiographic control 8 to 24 hours after graft reperfusion and left for 4 to 5 days; serum samples were taken at the same time-points as stated earlier. A radiograph performed daily was used to verify the correct location of the catheter.

All patients received 0.5 to 1 g of methylprednisolone during the anhepatic phase of LT. Thereafter, patients received 1 of the following immunosuppressive regimens: (1) cyclosporine A or tacrolimus associated with corticosteroids (13 patients); (2) tacrolimus, mofetil mycophenolate, and a monoclonal antibody anti-interleukin-2 receptor (7 patients).

Blood samples taken either from the peripheral circulation or from the hepatic veins were centrifuged within 2 to 3 hours after extraction, aliquoted, and frozen at -80°C . All patients gave their written informed consent before inclusion in the study protocol, which was approved by the Ethics Committee of our Center.

HCV-RNA Detection and Quantification. The concentration of HCV RNA was determined by using a quantitative reverse-transcription polymerase chain reaction (RT-PCR) assay (Cobas AmpliCor HCV Monitor 2.0; Roche Diagnostics, Branchburg, NJ), that achieves a sensitivity of approximately 600 IU/mL. The assay was performed according to the manufacturer's instructions. Samples with HCV-RNA concentration exceeding 800,000 IU/mL were diluted to 1/100 and retested. Samples belonging to the same patient were assayed in the same run, except for those that were retested.

Samples testing negative with the commercial assay were retested by a more sensitive in-house RT-PCR assay in 2 indepen-

Table 1. Primers Used to Amplify the 5' Untranslated Region of the HCV Genome

External sense primer	ACT GTC TTC ACG CAG AAA GCG TCT AGC CAT
External antisense primer	CGA GAC CTC CCG GGG CAC TCG CAA GCA CCC
Internal sense primer	ACG CAG AAA GCG TCT AGC CAT GGC GTT AGT
Internal antisense primer	TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG

dent experiments. Total RNA was extracted from 100 μL of serum with Trizol (GIBCO BRL Life Technologies, Barcelona, Spain), following the manufacturer's instructions. The RNA pellet was resuspended in 10 μL of RNase-free water containing 10 mmol/L dithiothreitol and 5% RNasin (20–40 U/ μL) (Promega, Madison, WI). After incubation of the RNA at 65°C for 2 minutes, complementary DNA synthesis was performed with avian myeloblastosis virus reverse transcriptase (AMV; Promega) and the external antisense primer (Table 1). Briefly, 10 μL of RNA were added to a master mix consisting of 2 μL of PCR buffer 10 \times , 2 μL of dNTP 10 mmol/L, 2 μL of MgCl_2 25 mmol/L, 3 μL of 10 $\mu\text{mol/L}$ external antisense primer, and 1 μL of AMV (total volume 20 μL). After incubation for 1 hour at 42°C , 56.5 μL of water were added and the mixture was incubated at 95°C for 5 minutes. Complementary DNA was then amplified with a nested PCR by amplification of the 5' untranslated region with nested primer pairs (Table 1).¹⁸ For amplification, a master mix consisting of 8 μL of PCR buffer 10 \times , 2 μL of 10 $\mu\text{mol/L}$ external antisense primer, 5 μL of 10 $\mu\text{mol/L}$ external sense primer, 8 μL of MgCl_2 25 mmol/L, and 0.5 μL of Taq Expand (Roche Diagnostics, Molecular Biochemicals, Barcelona, Spain) were added. The first round of PCR was performed for 35 cycles with denaturation at 94°C for 1 minute (initial denaturation step for 3 minutes), annealing at 45°C for 2 minutes, and amplification at 72°C for 3 minutes. For the second round of PCR amplification, a 10- μL aliquot of the first PCR was added to a master mix consisting of 9 μL of PCR buffer 10 \times , 2 μL of dNTP 10 mmol/L, 5 μL of 10 $\mu\text{mol/L}$ internal antisense primer, 5 μL of 10 $\mu\text{mol/L}$ internal sense primer, 9 μL of MgCl_2 25 mmol/L, 0.5 μL of Taq Expand, and 59.5 μL of water. The same cycling conditions were used for the second round of PCR. Two positive controls (HCV-RNA concentration 5 IU/mL and 500 IU/mL, respectively) and 4 negative controls were used in each reaction.

HCV Genotyping. HCV genotype was determined by restriction fragment length polymorphism analysis of the 5' untranslated region, as previously described.¹⁹

Statistical Analysis. Quantitative variables are expressed as mean (95% confidence interval [CI]). Viral load decay slopes during the anhepatic phase and after graft reperfusion were calculated by linear regression by using HCV-RNA concentrations expressed in natural logarithm. The elimination half-life of hepatitis C virions was calculated by using the equation $t_{1/2} = \ln(2)/\text{slope}$. The HCV doubling time in patients with increasing HCV-RNA concentrations were calculated by using the same formula. Comparison between qualitative variables were made by the Fisher's exact test. Linear regression was used to analyze a possible relationship between quantitative variables.

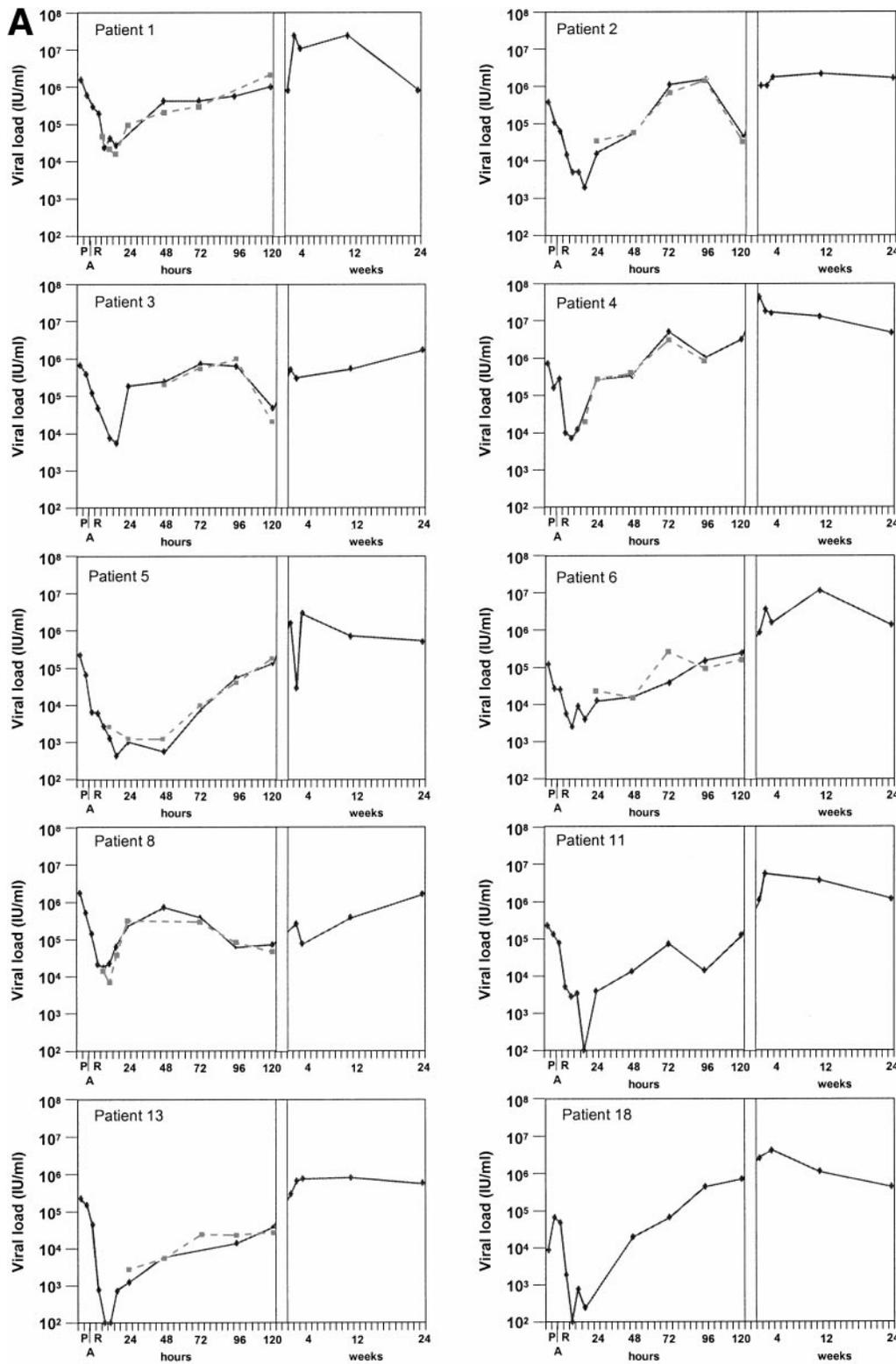


Fig. 1. HCV kinetics during and after liver transplantation. Patients are classified according to the viral kinetics pattern during the first days following graft reperfusion: (A) patients with rapid increase in HCV viral load ($\sim 2 \log_{10}$) or pattern 1.

Results

Viral Kinetics During the Anhepatic Phase. HCV RNA was quantified in serum samples obtained before transplantation and at the beginning and at the end of the anhepatic phase. During the anhepatic phase, HCV-RNA concentration decreased in 18 of 20 patients (mean decrease $0.48 \log_{10}$ IU/mL, 95% CI 0.29-0.68) and

remained practically constant in 2 patients (Figs. 1 and 2). The duration of the anhepatic phase ranged from 45 to 207 minutes. Assuming a first-order elimination kinetics, the mean viral load decay slope was -0.92 (95% CI -0.52 to -1.32) and the mean elimination half-life was 2.2 hours (95% CI 0.65-3.7) (Table 2). We did not find a relationship between the viral load decay (or decay slope) and the pretransplantation viral load or the duration

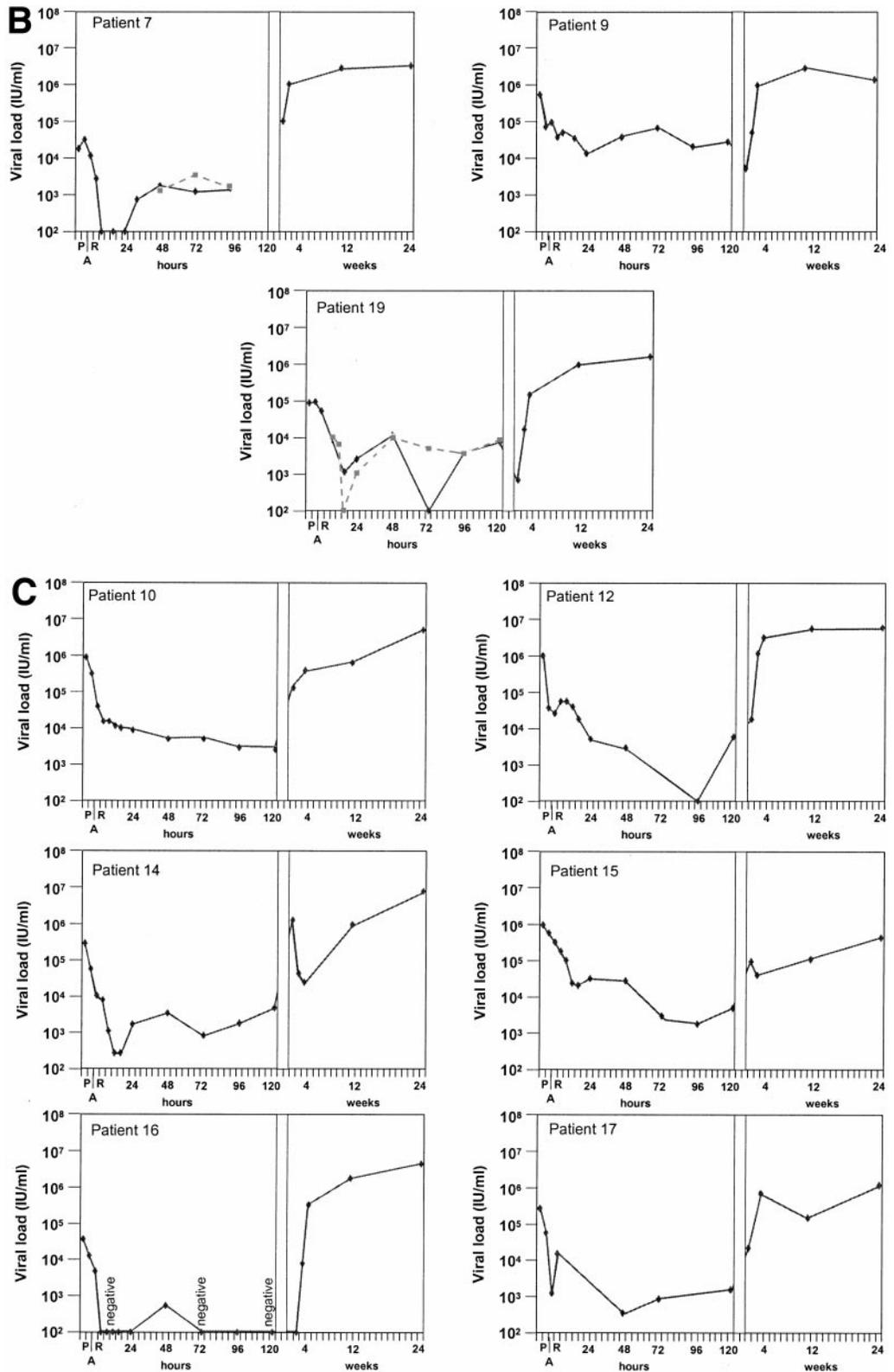


Fig. 1. (Cont'd.) (B) patients with unchanging viral load (or increase in viral load $< 1 \log_{10}$) or pattern 2; (C) patients with progressive decrease in HCV RNA or pattern 3. HCV-RNA concentrations are expressed in IU/mL and depicted in the y axis in a logarithmic scale. Time is represented in the x axis, in hours and weeks. Viral load in the systemic circulation is depicted with a **continuous line**; HCV viral load in the hepatic veins is depicted with a **discontinuous line**. Samples that tested negative by a sensitive in-house RT-PCR assay are indicated. P, pretransplantation; A, anhepatic phase; R, reperfusion phase.

of the anhepatic phase. We found, however, a significant correlation between the decrease in viral load and the following variables: the amount of blood loss during the anhepatic phase ($r = 0.78$, $P < .001$), the number of red blood cell concentrates transfused during the anhepatic phase ($r = 0.71$, $P = .001$), and the entire surgical procedure ($r = 0.76$, $P < .001$). A correlation

between the viral load decay slope and the amount of blood loss was also evident during the anhepatic phase ($r = 0.6$, $P = .02$). Considering the mean blood loss (2.5 L) and the mean transfusion requirements during surgery (red blood cell 6 U; plasma 1.5 L, plasma expanders 1.8 L), the calculated viral load decay slopes and elimination half-life values should be considered maximum HCV

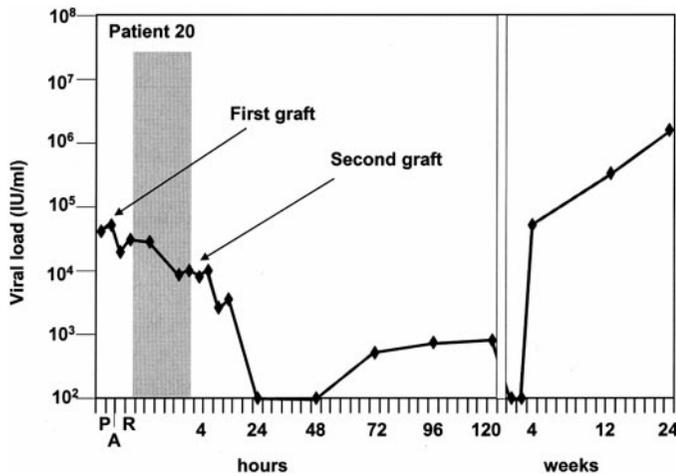


Fig. 2. HCV kinetics in a patient with a prolonged anhepatic phase. After implantation of the first graft the organ increased in size owing to difficult hepatic venous outflow and severe hemodynamic instability forced the surgeons to remove the liver and to perform a portocaval shunt. The patient was put on an urgent waiting list and received a second graft 20 hours later. Implantation of the second graft was uneventful. HCV-RNA concentrations are expressed in IU/mL and depicted in the y axis in a logarithmic scale. Time is represented in hours and weeks in the x axis. The prolonged anhepatic phase is shadowed.

extraction rates. Regarding the 2 patients in whom the viral load did not decrease during the anhepatic phase, we did not find any differences in the analyzed variables in comparison with the remaining patients.

We had the opportunity to study HCV kinetics in a patient with a prolonged anhepatic phase of 20 hours (patient 20). After implantation of the liver, the organ increased in size because of diffi-

cult hepatic venous outflow; severe hemodynamic instability forced the surgeons to remove the liver and to perform a portocaval shunt. The patient was put on an urgent waiting list and received a second graft 20 hours later. During this period the patient remained hemodynamically stable, with minimum transfusion requirements (2 U red blood cells and 0.5 L plasma). Serum samples were taken at the beginning, during, and at the end of this prolonged anhepatic phase and HCV-RNA concentrations were determined at each time-point (Fig. 2). In 20 hours, the HCV-RNA concentration decreased 0.58 log₁₀ IU/mL, following a first-order kinetics as deduced by linear regression (*r* = 0.95). The viral load decrease slope was -0.067 and the deduced elimination half-life of hepatitis C virions was 10.3 hours.

Viral Kinetics After Reperfusion of the Graft. HCV RNA was quantified in serum samples taken at 4, 8, 12, 16, and 24 hours after graft reperfusion and daily thereafter until day 5 posttransplantation. A sharp reduction in HCV-RNA concentration occurred after the reperfusion phase in all but 1 patient. HCV viral load reached its lowest level 8 to 24 hours after reperfusion (mean viral load decrease 1.53 log₁₀ IU/mL, 95% CI 1.22-1.85). By the quantitative test, HCV RNA became undetectable after graft reperfusion in 6 patients, 4 of them with pretransplant HCV-RNA concentrations below 10⁵ IU/mL (Fig. 1). However, when negative samples were retested by a more sensitive assay, HCV RNA was undetectable in only 1 patient (patient 16) at 3 time-points (Fig. 1). HCV viral load decay after graft reperfusion followed a first-order elimination kinetics, with a mean decay slope of -0.34 (95% CI -0.22 to -0.46). The mean elimination half-life of hepatitis C virions was 3.44 hours (95% CI 2.02-4.86) (Table 2). We did not find a relationship between the viral load decay (or decay slope) and the amount of blood loss or transfusion requirements during the reperfusion phase. In the patient with a pro-

Table 2. Epidemiologic and Virologic Features of 20 Consecutive Patients Undergoing LT for HCV-Related Cirrhosis

Patient Number	Age	Sex	Genotype	Viral Load Before LT*	Elimination t _{1/2} of HCV (hr) Anhepatic/Reperfusion	HCV Kinetics Pattern (HCV Doubling Time in hours)†	Immunosuppressive Regimen‡
1	54	M	1b	1,500,000	3.23/2.21	1 (20.7)	A
2	46	M	1b	373,000	1.40/3.48	1 (8.4)	A
3	55	M	2	667,000	0.59/3.45	1 (34.6)	A
4	59	F	1b	710,000	-/1.53	1 (7.3)	A
5	47	F	1b	221,000	0.22/3.96	1 (11)	A
6	56	M	1b	121,000	10.33/2.44	1 (19)	A
7	31	F	1a	17,700	0.79/1.16	2	A
8	63	F	1b	1,670,000	0.54/2.61	1 (7.4)	B
9	25	M	1a	566,000	-/6.73	2	A
10	65	F	1b	916,000	0.39/12.84	3	B
11	61	M	1a	219,000	1.72/2.03	1 (7)	B
12	62	M	1b	1,016,000	2.10/7.97	3	B
13	59	M	1b	231,000	0.47/0.91	1 (16.1)	A
14	56	M	1b	289,200	0.42/2.14	3	B
15	65	M	1b	966,000	1.62/3.71	3	B
16	43	M	No 1	38,200	0.99/0.71	3	B
17	59	M	1b	280,000	0.39/-	3	A
18	64	F	1b	8,920	2.22/0.90	1 (7.7)	A
19	65	F	1b	89,500	1.56/2.88	2	A
20	59	F	1b	41,300	10.3/3.75	2	A

*Viral load in IU/mL.

†HCV kinetics pattern 24 hours after graft reperfusion. 1, increase in viral load; 2, unchanging viral load; 3, decrease in viral load. HCV doubling time is shown in parentheses in patients with pattern 1.

‡A, cyclosporine A or tacrolimus associated with corticosteroids; B, tacrolimus, mofetil mycophenolate, and a MAb anti-IL2 receptor.

longed anhepatic phase of 20 hours, a sharp decrease in viral load ($1.99 \log_{10}$ IU/mL) occurred after reperfusion of the second graft (viral load decay slope -0.18), with a deduced HCV elimination half-life (3.75 hours) significantly shorter than that of the anhepatic phase (Fig. 2, Table 2). The transfusion requirements after reperfusion (2 U red blood cells and 0.5 L plasma) were similar to those during the prolonged anhepatic phase.

The HCV elimination half-life after graft reperfusion was unusually long in 3 patients (patients 9, 10, and 12). We did not find any remarkable differences between these 3 patients and the remaining individuals regarding pretransplantation viral load and transfusion requirements. In 2 of them, however, there was significant ischemia-reperfusion injury of the graft that caused a remarkable elevation of aspartate transaminase and alanine transaminase values ($>1,000$ IU/mL) within the first 24 to 48 hours. In the only patient in whom viral load did not decrease immediately after graft reperfusion (patient 17), ischemia-reperfusion injury was also remarkable.

After this phase of viral load decline, we observed 3 different HCV kinetic patterns during the first week after LT (Table 2). The first one (pattern 1) was characterized by a rapid increase in HCV viral load ($\sim 2 \log_{10}$). The second pattern (pattern 2) was identified by unchanging viral load (increase in viral load $<1 \log_{10}$). The third pattern (pattern 3) was characterized by a progressive decrease in HCV RNA. HCV-RNA concentrations increased rapidly to pretransplantation levels in 10 patients (Fig. 1A) with a viral load increase slope of 0.07 (95% CI 0.04-0.09) and a mean HCV doubling time of 13.8 hours (95% CI 6.6-21 hours). HCV RNA remained at similar levels in 4 patients (Figs. 1B and 2) and followed a second-phase decline in 6 patients (Fig. 1C). Interestingly, 5 of the 6 patients with a second-phase decline in HCV viral load were part of a group of 7 patients whose immunosuppressive regimen did not include corticosteroids (regimen B). In contrast, only 1 of the 13 patients who received corticosteroids as part of their immunosuppressive regimen showed a second-phase decline in HCV viral load ($P < .01$) (Table 2). Viral load kinetic patterns were not determined by pretransplantation HCV-RNA concentrations.

After the first week of transplantation, viral kinetics followed a similar pattern in most patients. We observed a progressive increase in viral load during weeks 2, 3, and 4 after LT, even in patients with viral kinetic patterns 2 and 3. In general, this increase in viral load coincided with higher and more stable levels of cyclosporine or tacrolimus. However, this was not the case in all individuals, such as in patients 12 and 19 (data not shown).

We did not find any significant differences in the early HCV kinetics among patients undergoing living-related (patients 4, 6, 9, and 13) or cadaveric liver transplantation (the remaining patients), or among patients infected with different HCV genotypes.

Viral Kinetics Based on Samples Taken From the Hepatic Veins. Previously published data suggested that HCV RNA remained undetectable in most patients up to 48 to 72 hours after liver transplantation.¹² To determine if HCV RNA could be detected earlier in blood drained directly from the liver, we catheterized the hepatic veins of 10 patients shortly after graft reperfusion. We did not find, however, significant differences between HCV-

RNA concentrations in the peripheral circulation and the hepatic veins and viral kinetics followed a similar pattern in both sites (Fig. 1).

Discussion

We studied viral kinetics in 20 patients undergoing LT for HCV-related disease. Our results show that removal of the infected liver causes a significant decrease in HCV-RNA concentration, which can be explained in part by the lack of virion production.¹² The decline in viral load during the anhepatic phase varied significantly from patient to patient, most likely because of the large number of variables that may influence viral load during surgery. Among these variables, we found a clear correlation between the decrease in viral load and the amount of blood loss and transfusion requirements during surgery, suggesting that the viral load decay values observed during this phase are maximum estimates. One of the limitations of the analysis is that only 1 sample at the beginning and at the end of a short anhepatic phase does not allow detection of the presence of 2 or more phases in the viral kinetics.

After the implantation of the new graft, HCV viral load continued to decrease exponentially in all but 1 patient. Although we did not find a relationship between the viral load decay and the transfusion requirements, we cannot exclude some contribution of this variable to the decrease in HCV-RNA concentrations. We hypothesize that after graft reperfusion, massive entrance of HCV into the hepatocytes or HCV uptake by the liver reticuloendothelial system is the cause, at least in part, of HCV clearance. Hepatic clearance of hepatitis C virions after graft reperfusion is strongly supported by the data obtained in 1 patient with a prolonged anhepatic phase of 20 hours. In this patient the elimination half-life of HCV was significantly longer during the anhepatic phase than after graft reperfusion. This indicates that viral clearance occurs relatively slowly in the absence of liver, whereas it increases significantly after the implantation of a graft. The lower clearance rates after graft reperfusion observed in a significant number of patients might be explained, in part, by active virus production after implantation of the new graft. Regarding the lack of significant differences between the viral load in the systemic circulation and the hepatic veins, several reasons may explain this finding. First, our study was not aimed to measure HCV hepatic clearance and we did not determine viral load in the portal vein. Second, hepatic vein catheterization immediately after transplantation was difficult and blood samples were not available for most patients during the viral load decay slope that followed graft reperfusion. Finally, it is possible that the massive hepatic blood flow minimizes slight differences in viral load. This is supported by the lack of significant differences in HCV-RNA concentrations between the hepatic veins and the systemic circulation during the sharp increase in HCV viral load that occurred the first days after LT.

The elimination half-life of hepatitis C virions during the anhepatic phase is somewhat shorter in our study than in the study published by Fukumoto et al.¹² However, HCV half-life in the latter study was based on the decrease in viral load during both the anhepatic and after reperfusion phases. In fact, the elimination half-life of HCV after graft reperfusion in our study is similar to that reported by Fukumoto et al.¹² and other studies.^{17,20} In 3 patients, the HCV elimination half-life was unusually long (over 6

hours), and in 2 of them ischemia-reperfusion injury of the graft was remarkable. It is possible that ischemia-reperfusion damage of hepatocytes impairs HCV entrance and prolongs the elimination half-life of circulating virions. This might also explain the lack of viral load decay immediately after graft reperfusion in 1 patient.

Differently from previous reports,¹² we found that HCV RNA remained detectable in almost all patients after LT. These differences are most likely explained by the use of a more sensitive test to detect HCV RNA. Our data indicate that virus particles are constantly present in the blood stream during the anhepatic phase and cause infection of the new graft.

HCV viral load increased as soon as 12 hours after graft reperfusion, reaching pretransplantation levels by day 4 after transplantation in a significant proportion of patients. The rapid increase in HCV viral load indicates that viral replication is highly efficient after LT and proves the high capacity of HCV to adapt to a completely new environment. Not in all patients, however, did HCV kinetics follow the same pattern. In 6 patients, HCV-RNA concentrations continued to decline during the first days after LT. Differences in the immunosuppressive regimen appeared to influence HCV kinetics immediately after LT. In fact, HCV-RNA concentrations increased rapidly in patients receiving corticosteroids as part of the immunosuppressive therapy,²¹⁻²³ whereas it continued to decrease in most patients in whom corticosteroids were not part of the immunosuppression therapy. Although this observation needs to be confirmed in other studies, it is possible that some immunosuppressive regimens might be more appropriate if early antiviral therapy to eradicate HCV is considered.

In general, we found that HCV-RNA concentrations increased progressively after the first week of transplantation and reached a plateau by the first month. Apart from the possible effect of the immunosuppression on HCV-RNA concentrations, other variables might influence this increase in viral load. It is possible that the HCV quasispecies populations require some time to reach an equilibrium in a context of a preestablished cellular and humoral immune response.²⁴

Based on our data on HCV kinetics, it seems clear that any immunoprophylaxis attempt should start during the anhepatic phase, before circulating hepatitis C virions can infect the hepatocytes of the new graft. Regarding antiviral therapy, it might be more efficient if initiated during or immediately after LT, when viral load reaches its lowest level. It would be relevant to analyze if this therapeutic strategy is more efficient than the current antiviral preemptive regimens beginning a few weeks after LT.⁹⁻¹¹

In summary, in patients undergoing LT, HCV viral load decreases during the anhepatic phase and after graft reperfusion because of lack of virus production, blood loss, and hepatic viral clearance. Despite the viral load decay, hepatitis C virions continue to circulate and infect the new graft rapidly. HCV replication in the liver graft begins as soon as a few hours after LT in most patients. These results might be helpful to design more efficient therapeutic strategies to eradicate HCV early after LT.

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