Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

No evidence of occult hepatitis C virus (HCV) infection in serum of HCV antibody-positive HCV RNA-negative kidney-transplant patients

Florence Nicot,[1,2] Nassim Kamar,[3,4,5] Bernard Mariamé,[1] Lionel Rostaing,[1,3,4] Christophe Pasquier[1,2,3] and Jacques Izopet[1,2,3]

1 INSERM, U563, Toulouse, France
2 Department of Virology, CHU Toulouse, Purpan Hospital, Toulouse, France
3 Université Toulouse III Paul-Sabatier, Centre de Physiopathologie de Toulouse Purpan, Toulouse, France
4 Department of Nephrology, Dialysis and Multiorgan Transplantation, CHU Toulouse, Rangueil Hospital, Toulouse, France
5 INSERM, U858, Toulouse, France

Keywords
hepatitis C virus, kidney transplantation, occult HCV, persistence

Correspondence
Prof. Jacques Izopet, Laboratoire de Virologie, Institut Fédératif de Biologie, CHU Toulouse, 330 avenue de Grande-Bretagne, TSA40031, Toulouse 31059, France. Tel.: 33 5 67 69 04 24; fax: 33 5 67 69 04 25; e-mail: izopet.j@chu-toulouse.fr

Received: 23 September 2009
Revision requested: 26 October 2009
Accepted: 17 November 2009
Published online: 11 December 2009

doi:10.1111/j.1432-2277.2009.01025.x

Summary

Persistence of hepatitis C virus (HCV) in patients who cleared HCV is still debated. Occult HCV infection is described as the presence of detectable HCV RNA in liver or peripheral blood mononuclear cells (PBMCs) of patients with undetectable plasma HCV-RNA by conventional PCR assays. We have assessed the persistence of HCV in 26 kidney-transplant patients, followed up for 10.5 years (range 2–16), after HCV elimination while on hemodialysis. If HCV really did persist, arising out of the loss of immune control caused by institution of the regimen of immunosuppressive drugs after kidney transplantation, HCV reactivation would have taken place. Their immunosuppression relied on calcineurin inhibitors (100%), and/or steroids (62%), and/or antimetabolites (94%). An induction therapy, given to 22 patients, relied on rabbit antithymocyte globulin (59%) or anti-IL2-receptor blockers (32%). All patients had undetectable HCV RNA as ascertained by several conventional tests. At the last follow-up, no residual HCV RNA was detected in the five liver biopsies, the 26 plasma, and in the 37 nonstimulated and 24 stimulated PBMCs tested with an ultrasensitive RT-PCR assay (detection limit, 2 IU/ml). No biochemical or virologic relapse was seen during follow-up. The absence of HCV relapse in formerly HCV-infected immunocompromised patients suggests the complete eradication of HCV after its elimination while on dialysis.

Introduction

The prevalence of anti-hepatitis C virus (HCV) antibodies in patients undergoing regular dialysis is consistently higher than in the general population, ranging from 7% to 40% [1–3]. In French hemodialysis units, it has decreased by 7.7% during the past few years but HCV infection still occurs and necessitates appropriate management. After kidney transplantation, patient survival is lower in HCV-positive as compared with matched HCV-negative kidney-transplant patients [4]. Because of the relatively high rate of sustained virologic response (SVR) in HCV-positive dialysis patients treated by anti-HCV therapy, it has been recommended to treat all kidney-transplant candidates with α-interferon [5]. Furthermore, it has been previously shown that, when sustained HCV RNA clearance occurs in dialysis patients, no relapse is observed after transplantation, despite chronic immunosuppressive treatment [6].

Very recently, the presence of genomic HCV RNA in peripheral blood mononuclear cells (PBMCs) has been found in 49 out of 109 (45%) serum HCV antibody-negative/HCV RNA-negative hemodialysis patients with

© 2009 The Authors

abnormal liver-enzyme levels [7]. This is defined as 'occult HCV infection' in dialysis patients. Occult HCV infection is a new entity described as HCV-genome RNA that is not detected in plasma using conventional PCR assays, but which can be detected in liver tissue, PBMCs [8], and even plasma [9] using a highly sensitive test. Low concentrations of HCV genomic RNA have been detected in PBMCs, lymphocytes, macrophages, dendritic cells, and the livers of immunocompetent patients who are cleared of HCV either spontaneously [10,11] or after treatment [12–14]. Thus, this could have a big impact on the management of hemodialysis patients in dialysis units. Kidney-transplant patients lose immune control because of institution of the regimen of immunosuppressive drugs. In this setting, if HCV infection really does persist at low levels in plasma or PBMCs, we hypothesize that it should be more easily detectable than in immunocompetent patients.

Since 2006, we have used ultrasensitive assays for HCV RNA to systematically look for the persistence of HCV RNA in the plasma and PBMCs of formerly HCV-infected patients receiving immunosuppressive therapy for kidney transplantation.

Methods

Study population

All anti-HCV antibody positive/RNA negative kidney-transplant patients were from the Department of Nephrology, Dialysis and Multi-Organ Transplantation of Toulouse University Hospital, and had attended outpatient and inpatient clinics between May 2006 and December 2008 ($n = 26$). All had recovered from HCV infection while on dialysis, either spontaneously ($n = 10$) or after anti-HCV treatment ($n = 16$). HCV infection was defined as anti-HCV antibody-positive serum. The plasma from these patients repeatedly tested HCV RNA-negative using the conventional real-time RT-PCR COBAS™ Ampliprep/COBAS™ Taqman HCV test (CAP/CTM; Roche Diagnostics, Meylan, France), with a detection limit of 15 IU/ml. When sera from the viremic phase was available ($n = 10$), HCV genotype was determined by sequencing a 382 nt fragment within the NS5B region [15]. Serotyping of the 16 remaining patients with no available viremic frozen samples was done using Murex HCV serotyping™ kit (Abbott Murex, Rungis, France). This study used blood samples collected between 2 and 16 years after HCV was cleared from their serum.

Blood collection

Four ethylene diamine tetra acetic acid (EDTA) tubes were collected from each patient, after they had given their informed consent. The study was conducted conforming to the Declaration of Helsinki.

Isolation of PBMCs and *in vitro* stimulation

Peripheral blood mononuclear cells were isolated from blood by density-gradient centrifugation (Lymphoprep, Abcys, France). The isolated PBMCs were divided into two parts: one was immediately frozen at −80 °C (unstimulated PBMCs) and the other was cultured (stimulated PBMCs). Stimulation of cells in culture increases HCV RNA detection in PBMCs from patients who are apparently HCV RNA-negative [16]. Cells (1×10^7 million) were washed and suspended in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, Cergy-Pontoise, France) supplemented with 20% heat-inactivated fetal bovine serum (SVF) (Invitrogen) and interleukin-2 (IL-2) at 20 U/ml (Proleukin®, Roche). They were cultured for 48 h, and then phytohemagglutinin (PHA, 5 μg/ml, Remel, Santa-Fe, NM, USA) was added and culture was continued for an additional 72 h. The cells were then separated by centrifugation. Samples of approximately 5×10^6 (3.5×10^6 to 6×10^6) of unstimulated or stimulated PBMCs were preserved at −80 °C for analysis.

Ultrasensitive detection of HCV RNA in plasma

Hepatitis C virus-RNA was extracted from 1 ml plasma with the QIAmp UltraSens Virus kit (Qiagen, Courtaboeuf, France), following the manufacturer's instructions. The extracted RNA was precipitated with ethanol, suspended in RNase-free water, and placed into a single reaction to obtain maximum sensitivity. HCV RNA was detected by amplification of the 5′ untranslated region (UTR) region of the HCV genome. The following primer pairs were used: 5′-CTCGCAAGCACCCTATCAG GCAGT-3′ (antisense, KY-78) and 5′-GCAGAAAGCGTC TAGCCATGGCGT-3′ (sense, KY-80) for the RT-PCR round; and 5′-CGGGAGAGCCATAGTGG-3′ (sense, R-130) and 5′-CGGGAGAGCCATAGTGG-3′ (antisense, R-290) for the nested round. RT-PCR was performed with the Superscript III one-step PCR (Invitrogen, Carlsbad, NM, USA). The RT-PCR conditions were: reverse transcription at 60 °C for 30 min, followed by initial denaturation at 94 °C for 2 min, and by 55 cycles of 94 °C for 15 s, 68 °C for 30 s, and 68 °C for 30 s. The sensitivity of the nested one-step RT-PCR amplification was determined by testing stepwise dilutions of a quantified HCV RNA plasma (the second World Health Organization standard for HCV RNA, from National Institute for Biological Standards and Control). The low HCV RNA values (10, 5, 2, and 1 IU/ml) were tested in triplicates. The detection limit was 2 IU/ml (or five copies/ml). Negative

© 2009 The Authors

controls (plasma from uninfected patients) and positive controls (plasma from chronically HCV-infected patients) were simultaneously tested in each run.

Detection of HCV-RNA in PBMCs and liver biopsies by nucleic acid hybridization assay

Hepatitis C virus-RNA was extracted from about 5×10^6 cells or 5–30 mg of frozen liver tissue stored at −80 °C, using the RNeasy minikit (Qiagen), following the manufacturer's instructions. The total RNA extracted from PBMCs or liver biopsies was precipitated with ethanol, suspended in RNase-free water, and was used in a single reaction to maximize sensitivity. HCV RNA was detected by amplifying the 5′ UTR region of the HCV genome by RT-PCR, followed by a nested PCR (as for the plasma). Negative controls, i.e., PBMCs or liver biopsies taken from uninfected individuals, and positive controls, i.e., PBMCs or liver biopsies taken from chronically HCV-infected patients, were simultaneously tested in each run. The specificity of PCR amplicons and the validity of the detection were confirmed by Southern blotting using ^{32}P-labeled (Perkin-Elmer SAS, France) HCV fragments as a probe. This probe was generated by transcribing RNA isolated from a chronically HCV-infected patient. The cDNA was amplified with the sense primer Rad-S (5′-(A/G)A(C/T)CACTCCCCTGTGAGGAAC-3′) and reverse primer Core-506 (5′-TCT ACC TCG AGG TTG CGA-3′). The final 517-bp product was purified and cloned into the promoter pCR4 plasmid vector using the TOPO TA cloning kit for sequencing (Invitrogen). The probe was labeled with ^{32}P using the DecaLabel DNA labeling kit (Fermentas, Saint-Rémy-Lès-Chevreuses, France), following the manufacturer's instructions. The detection limit was 2 IU/reaction (or five copies/reaction).

Results

Patients' characteristics

The patients' characteristics are summarized in Table 1. Ten (39%) patients had spontaneous clearance of HCV and 16 (61%) were cleared of HCV after interferon-α (IFN-α) ($n = 15$) or PEG-IFN-α ($n = 1$) therapy while on hemodialysis. The median follow-up time after HCV RNA clearance was 10.5 years (range 2–16 years). Seventeen patients who had been infected with HCV had the following: HCV genotype 1 ($n = 12$), genotype 2 ($n = 2$), genotype 3 ($n = 1$) or genotype 4 ($n = 1$). Genotype was not ascertained for the other nine patients (four who were spontaneously cleared of the virus and five who were therapeutically cleared of the virus) as they had undetectable or very low levels of anti-HCV antibodies. No patient was coinfected with human immunodeficiency virus (HIV). Four patients had positive HBs antigens (HBV+), and three of these four had positive plasma HBV DNA.

Table 1. Patients' characteristics.

Parameter	
Gender (M/F)	19/7
Age, years (range)	50 (31–66)
Time since KT, months (range)	59 (1–224)
HCV treatment	
No	10
Yes	16
Time since HCV RNA clearance, years (range)	10.5 (2–16)
Primary kidney disease	
GN	15
CIN	5
PKD	4
Diabetes mellitus	1
Nephroangiosclerosis	1
Induction therapy	22
RATG	13
Anti-IL2R	8
RATG + rituximab	1
Immunosuppressive treatment (*n*, %)	
Cyclosporin A	9 (35)
Tacrolimus	14 (54)
Mycophenolic acid	20 (79)
Sirolimus	2 (8)
Azathioprine	4 (15)
Steroids	16 (62)
Belatacept	1 (4)
Acute rejection (*n*, %)	8 (31)
Rejection therapy	
Steroids	4
RATG	3
OKT3	1

M, male; F, female; IFN, interferon; GN, glomerulonephritis; CIN, chronic interstitial nephropathy; PKD, polycystic kidney disease; RATG: rabbit antithymocytes globulins; anti-IL-2R: interleukin 2 receptor blockers; KT, kidney transplantation; HCV, hepatitis C virus.

At last follow-up, i.e., at last blood collection, median serum aspartate aminotransferase (AST) was 18 IU/l (range, 7–63 IU/l), alanine aminotranferase (ALT) was 21 IU/l (range, 9–84 IU/l), and gamma glutamyl transpeptidase (γ-GT) was 23 IU/l (range, 11–85 IU/l) (Table 2). The abnormal enzyme values observed in some patients (Table 2) can be explained by active HBV infection (patient 8), hepatic polycystic disease (patient 12), alcohol consumption (patient 20) and everolimus intake (patient 26). Four out of the 10 patients who spontaneously eliminated HCV have a liver biopsy necessitated by HBV coinfection. For the 16 patients who were cleared of HCV after treatment, four had a post-treatment liver biopsy: A0F1 before treatment and A1F1 at 8 months after the end of treatment for the first patient, A1F1 before treatment and A1F1 at 35 months after the end of

© 2009 The Authors

Table 2. HCV RNA in the plasma and PBMCs of patients with cleared HCV infection.

Patient/Gender	HCV serology	Liver function test (IU/l) Last follow-up			Ultrasensitive HCV RNA in plasma	HCV RNA in PBMCs		Time between KT and HCV RNA testing (months)	Time between HCV RNA clearance and HCV RNA testing (years)
	Before KT/after KT	ALT (n = 5–45)	AST (n = 3–35)	γ-GT (n = 11–60)		Unstimulated	Stimulated		
1/F	+/+	19	22	14	–	– (2)	– (1)	56	11
2/M	+/+	17	20	37	–	– (2)	– (1)	4	5
3/M	+/+	20	21	22	–	– (2)	– (2)	133	13
4/F	+/+	7	14	22	–	– (1)	– (1)	1	5
5/M	+/+	44	28	27	–	– (2)	– (2)	180	12
6/M	+/+	11	15	17	–	– (1)	– (1)	10	4
7/M	+/+	26	20	21	–	– (2)	– (1)	52	14
8/M	+/+	46	35	24	–	– (1)	– (1)	131	14
9/M	+/–	20	26	23	–	– (1)	– (1)	60	10
10/M	+/+	14	13	11	–	– (2)	– (1)	59	7
11/F	+/+	26	31	15	–	– (1)	– (1)	20	5
12/F	+/–	15	14	85	–	– (2)	– (2)	59	14
13/F	+/+	18	17	27	–	– (2)	– (2)	123	16
14/F	+/+	9	9	40	–	– (2)	– (1)	12	7
15/M	+/+	24	12	23	–	– (1)	ND	224	13
16/M	+/+	18	21	16	–	– (2)	– (2)	99	13
17/M	+/+	16	25	23	–	– (1)	– (1)	17	3
18/M	+/+	19	22	20	–	– (1)	– (1)	71	12
19/M	+/+	12	16	20	–	– (2)	– (2)	9	10
20/M	+/+	63	63	58	–	– (1)	– (1)	93	9
21/M	+/–	32	21	41	–	– (1)	– (1)	101	10
22/M	+/+	16	18	28	–	– (1)	ND	26	3
23/F	+/+	18	21	18	–	– (1)	– (1)	117	13
24/M	+/–	13	15	15	–	– (1)	– (1)	82	11
25/M	+/+	16	17	49	–	– (1)	– (1)	2	2
26/M	+/+	45	53	163	–	– (1)	– (1)	16	16

F, female; M, male; KT, kidney transplantation; AST, aspartate aminotranferase; ALT, alanine aminotransferase; γ-GT, gamma glutamyl transpeptidase; PBMC, peripheral blood mononuclear cell; HCV, hepatitis C virus.
Values just underneath AST, ALT and γ-GT indicate normal range for male and female.
Number in brackets indicates the number of PBMC samples tested.

treatment for the second patient, A1F0 at 23 months after the end of treatment for the third patient, and the fourth patient had no liver biopsy before treatment but had A0F0 liver biopsy at 27 months after the end of treatment. Thus no patient had a worse liver histology after treatment.

The median last collection time at post-transplantation follow-up was 59 months (range 1–224 months). After transplantation, eight patients experienced a biopsy-proven acute rejection. Four were successfully treated with steroid pulses and the remaining four patients experienced steroid-resistant acute rejection episodes that required either rabbit antithymocyte globulins (RATGs) ($n = 3$) or anti-CD3 monoclonal antibodies (OKT3) ($n = 1$). None of the patients experienced an acute antibody-mediated rejection. At last blood collection, serum-creatinine level was 139 μmol/l (range 94–273 μmol/l). Three out of 25 nondiabetic patients at transplantation (12%) developed post-transplantation diabetes mellitus: all three had received a tacrolimus-based immunosuppression. Proteinuria was absent in all but two patients, i.e., 0.5 and 0.8 g/day. No patient experienced any post-transplantation lymphoproliferative disorder, or an HCV-related *de novo* glomerulonephritis.

HCV RNA detection in plasma

Hepatitis C virus-RNA was repeatedly tested in the plasma of all patients using the COBAS™ AmpliPrep/COBAS™ TaqMan HCV test (CAP-CTM, detection limit of 15 IU/ml). In 23 patients (88%), HCV RNA was tested at least three times with a mean number of five samples per patient (range, 1–15). None of the plasma samples taken during the 16-year period after HCV clearance had detectable HCV genome RNA as assessed by conventional

© 2009 The Authors

techniques. No patient experienced a virologic relapse during post-transplantation follow-up.

The plasma samples taken at the last blood collection were also tested using an in-house ultrasensitive RT-PCR assay with a detection limit of 2 IU/ml. No residual HCV genome RNA was detected in any of the plasma samples from the 26 patients (Table 2).

HCV RNA detection in PBMCs and liver biopsies

Peripheral blood mononuclear cells were collected from all patients at least once. A second PBMC sample was taken from 11 of the 26 patients at between 2 and 23 months after the first sample. A total of 37 PBMCs samples were tested for HCV RNA using an in-house ultrasensitive RT-PCR assay, followed by Southern blotting (detection limit, 2 IU/reaction). Furthermore, 24 PBMC samples (65%) were stimulated in culture before testing for HCV RNA. None of the PBMC samples had detectable HCV RNA (Table 2).

Liver biopsies from five patients were tested for the presence of HCV RNA after they had recovered from HCV: four biopsies were from the four patients who spontaneously resolved their HCV infection and were coinfected with HBV and the last one from one of the four patients who had a liver biopsy after having HCV clearance induced by interferon-based therapy. None of the liver biopsy samples had detectable HCV RNA by 8 years (range: 2–12) after HCV clearance.

Discussion

Interferon-α is contraindicated after kidney transplantation because of the high risk of acute rejection [17]. However, it has been recently suggested that pegylated interferon-α-based treatment could be considered late after kidney transplantation [18]. In the recent Kidney Disease: Improving the Global Outcomes (KDIGO) guidelines [5], it has been recommended to treat all HCV-positive/RNA-positive patients who are candidates for a kidney transplantation with α-IFN. In this setting, a SVR is observed in approximately 40% of treated patients. It has been recently suggested that apparently cured patients may have an occult HCV infection [9,13,14,19] attributable to persistent low-level HCV replication. In a previous study, we did not observe any relapse of HCV replication after kidney transplantation in patients who were cleared of HCV RNA after IFN-α therapy while on dialysis [6]. In this study, we looked for the persistence of HCV in immunocompromised kidney-transplant patients who were cleared of the virus while on dialysis. In this very favorable situation for viral replication, we did not observe any relapse of HCV infection after a long-term follow-up (median, 10.5 years) despite intensive immunosuppressive therapy. In addition, using very sensitive virologic tools, we failed to detect any residual HCV RNA in the liver, the plasma or the PBMCs of the 26 immunocompromised patients.

Recently, Barril *et al.* [7] have detected occult HCV infection in 45% of antibody-negative/RNA-negative patients receiving dialysis with abnormal liver function. Although it may be of interest, these results should be interpreted with caution because of potential major concerns [20]. First, in their study, liver-enzyme levels were abnormal in patients experiencing occult HCV infection whereas, in dialysis patients with chronic active HCV infection, liver-enzyme levels were often within the normal range [21,22]. Second, they reported a very high proportion of deaths (39%) during the short length of follow-up. However, these deaths were not related to HCV-liver disease. This suggests the presence of another underlying disease other than HCV infection that may have been responsible for the increased enzyme levels. Third, seven of these hemodialysis patients had received a kidney transplant, but their serum HCV RNA remained negative after kidney transplantation. In HCV RNA-positive patients, there is a significant increase in serum HCV RNA concentration after transplantation because of the loss of HCV-immune control under immunosuppression [23–26]. Hence, it is surprising that no HCV RNA was detected in the serum of the seven kidney-transplant patients who had an occult HCV infection before transplantation. The number and/or type of immunosuppressive therapies were not reported in the Barril *et al.* study [7]. In our study, 13 patients (50%) received RATG or OKT3, and 20 patients (79%) received mycophenolic acid. Both drugs have been found to increase HCV viremia in HCV-infected patients [26–28] but none of the 26 kidney-transplant patients had a detectable HCV RNA in their plasma, which confirms and extends the results obtained in our previous study [6]. Finally, interestingly, none of our patients developed any HCV-related glomerulopathy or a liver disease within this long follow-up. No patients who had a post-treatment liver biopsy showed a deteriorating liver histology. Only three patients, who received a tacrolimus-based therapy, developed post-transplant diabetes mellitus. The occurrence of post-transplant diabetes mellitus in HCV-positive/RNA-positive patients is usually much more frequent [29]. HCV infection has also been identified as one of the important risk factors for tuberculosis in kidney-transplant patients [30]. In this study, none of the patients developed tuberculosis after transplantation.

Our findings strengthen the results obtained from recent studies that have detected no HCV genomic RNA in the plasma or PBMCs of 156 successfully treated

© 2009 The Authors

immunocompetent patients [31], or in the PBMCs of 69 anti-HCV-positive/HCV RNA-negative blood donors [32]. The absence of detectable HCV RNA genome in liver biopsies, PBMCs, and plasma of patients with impaired immunity indicates no persisting HCV. However, other studies, in smaller cohorts of patients (15–24 patients), have found PBMCs to be the site of HCV infection after successful treatment or spontaneous elimination of HCV infection in about 50% of patients [10,13,14,19,33]. The disagreement between these studies, i.e., either HCV elimination or HCV persistence, may be linked to differences within the criteria used to define plasma viremia in patients with apparent SVR. Indeed, standardized techniques used to classify patients as SVR differ between studies, ranging from 25 to 1000 copies/ml. About 60% of patients with an occult HCV infection had detectable HCV RNA virus in their plasma and their mean viral load was 71 HCV RNA copies/ml (range 18–192) [9]. Therefore, HCV genomic RNA would have probably been detected by conventional tests if sensitive RT-PCR assays [CAP/CTM or transcription-mediated assay (TMA)] had been used to screen these patients. Based on the previous published data [7,9,10,13], HCV RNA could have been detected in the PBMCs of 13 patients (50%) and in the plasma of seven patients (37%) in our cohort. Despite a small number of patients, the size of the studied population seems adequate to detect the presence of the HCV RNA genome in PBMCs or plasma. Data on fibrosis after HCV clearance were not available in all patients. The use of liver stiffness or fibrotest in this population for diagnosing liver fibrosis could be an acceptable alternative for future studies [34].

There are several reasons why HCV persistence is unlikely. First, HCV is an RNA virus that has no latent stage in its replication cycle and its genome cannot persist as DNA, unlike viruses such as HIV, HBV and herpes viruses. Second, long-term follow-up studies have shown that HCV genomic RNA rarely reappears in the plasma after SVR [35–37]. More recently, the clinical, virologic, and biochemical outcomes of a cohort of 150 patients with SVR followed for 5 years have not shown conclusive evidence of a virologic relapse [38]. A few cases of HCV recurrence in SVR patients who received immunosuppressive treatment have been reported supporting the hypothesis of HCV persistence. One patient was reinfected by the same HCV genotype after chemotherapy for lymphoma [39]. Lin *et al.* [40] reported the re-emergence of HCV in a patient who had received a short course of prednisone 7 months after the end of HCV therapy, and in another who underwent a kidney transplantation 7 months after the end of HCV therapy. All these patients were reinfected by the same genotype, but no accurate phylogeny was done on the hypervariable region of the HCV genome (e.g., region HVR1) to confirm that it was the same virus [41] or to exclude the possibility of reinfection from the same source as the first infection, or that they had a similar virus. This must be borne in mind in studies describing late relapse.

This report deals with kidney-transplant recipients, with cleared HCV infection, receiving subsequent immunosuppressive treatment which is usually thought to be responsible for a boost in HCV replication. We found no evidence of a persisting HCV reservoir in liver biopsies, PBMCs and plasma using an ultra-sensitive RT-PCR assay. The absence of a HCV relapse in formerly HCV-infected immunocompromised patients suggests the complete elimination of HCV while on dialysis.

Authorship

F. Nicot performed research, collected data and wrote the paper. B. Mariamé gave technical assistance. L. Rostaing and N. Kamar did the follow-up of patients and participated in paper writing. C. Pasquier and J. Izopet designed the study and participated in paper writing.

References

1. Finelli L, Miller JT, Tokars JI, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial* 2005; **18**: 52.
2. Fissell RB, Bragg-Gresham JL, Woods JD, *et al.* Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2004; **65**: 2335.
3. Jadoul M, Poignet JL, Geddes C, *et al.* The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. *Nephrol Dial Transplant* 2004; **19**: 904.
4. Kamar N, Ribes D, Izopet J, Rostaing L. Treatment of hepatitis C virus infection (HCV) after renal transplantation: implications for HCV-positive dialysis patients awaiting a kidney transplant. *Transplantation* 2006; **82**: 853.
5. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. *Kidney Int Suppl* 2008; **109**: S1.
6. Kamar N, Toupance O, Buchler M, *et al.* Evidence that clearance of hepatitis C virus RNA after alpha-interferon therapy in dialysis patients is sustained after renal transplantation. *J Am Soc Nephrol* 2003; **14**: 2092.
7 Barril G, Castillo I, Arenas MD, *et al.* Occult hepatitis C virus infection among hemodialysis patients. *J Am Soc Nephrol* 2008; **19**: 2288.
8. Carreno V, Bartolome J, Castillo I, Quiroga JA. Occult hepatitis B virus and hepatitis C virus infections. *Rev Med Virol* 2008; **18**: 139.

© 2009 The Authors

9. Bartolome J, Lopez-Alcorocho JM, Castillo I, *et al.* Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *J Virol* 2007; **81**: 7710.
10. Carreno V, Pardo M, Lopez-Alcorocho JM, Rodriguez-Inigo E, Bartolome J, Castillo I. Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. *J Infect Dis* 2006; **194**: 53.
11. Radkowski M, Horban A, Gallegos-Orozco JF, *et al.* Evidence for viral persistence in patients who test positive for anti-hepatitis C virus antibodies and have normal alanine aminotransferase levels. *J Infect Dis* 2005; **191**: 1730.
12. Castillo I, Rodriguez-Inigo E, Lopez-Alcorocho JM, Pardo M, Bartolome J, Carreno V. Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. *Clin Infect Dis* 2006; **43**: 1277.
13. Pham TN, MacParland SA, Mulrooney PM, Cooksley H, Naoumov NV, Michalak TI. Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. *J Virol* 2004; **78**: 5867.
14. Radkowski M, Gallegos-Orozco JF, Jablonska J, *et al.* Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Hepatology* 2005; **41**: 106.
15. Sandres-Saune K, Deny P, Pasquier C, Thibaut V, Duverlie G, Izopet J. Determining hepatitis C genotype by analyzing the sequence of the NS5b region. *J Virol Methods* 2003; **109**: 187.
16. Pham TN, Macparland SA, Coffin CS, Lee SS, Bursey FR, Michalak TI. Mitogen-induced upregulation of hepatitis C virus expression in human lymphoid cells. *J Gen Virol* 2005; **86**: 657.
17. Rostaing L, Izopet J, Baron E, Duffaut M, Puel J, Durand D. Treatment of chronic hepatitis C with recombinant interferon alpha in kidney transplant recipients. *Transplantation* 1995; **59**: 1426.
18. Pageaux GP, Hilleret MN, Garrigues V, *et al.* Pegylated interferon-alpha-based treatment for chronic hepatitis C in renal transplant recipients: an open pilot study. *Transpl Int* 2009; **22**: 562.
19. Gallegos-Orozco JF, Rakela J, Rosati MJ, Vargas HE, Balan V. Persistence of hepatitis C virus in peripheral blood mononuclear cells of sustained viral responders to pegylated interferon and ribavirin therapy. *Dig Dis Sci* 2008; **53**: 2564.
20. Kamar N, Nicot F, Izopet J, Rostaing L. Occult hepatitis C virus infection in hemodialysis patients: examining the evidence. *Am J Kidney Dis* 2009; **54**: 10.
21. Espinosa M, Martin-Malo A, Alvarez de Lara MA, Soriano S, Aljama P. High ALT levels predict viremia in anti-HCV-positive HD patients if a modified normal range of ALT is applied. *Clin Nephrol* 2000; **54**: 151.
22. Fabrizi F, Lunghi G, Andrulli S, *et al.* Influence of hepatitis C virus (HCV) viraemia upon serum aminotransferase activity in chronic dialysis patients. *Nephrol Dial Transplant* 1997; **12**: 1394.
23. Gane EJ, Naoumov NV, Qian KP, *et al.* A longitudinal analysis of hepatitis C virus replication following liver transplantation. *Gastroenterology* 1996; **110**: 167.
24. Kneteman NM. Steroid-free immunosuppression: balancing efficacy and toxicity. *Liver Transpl* 2001; **7**: 698.
25. Pereira BJ, Levey AS. Hepatitis C virus infection in dialysis and renal transplantation. *Kidney Int* 1997; **51**: 981.
26. Rostaing L, Izopet J, Sandres K, Cisterne JM, Puel J, Durand D. Changes in hepatitis C virus RNA viremia concentrations in long-term renal transplant patients after introduction of mycophenolate mofetil. *Transplantation* 2000; **69**: 991.
27. Nelson DR, Soldevila-Pico C, Reed A, *et al.* Anti-interleukin-2 receptor therapy in combination with mycophenolate mofetil is associated with more severe hepatitis C recurrence after liver transplantation. *Liver Transpl* 2001; **7**: 1064.
28. Zekry A, Gleeson M, Guney S, McCaughan GW. A prospective cross-over study comparing the effect of mycophenolate versus azathioprine on allograft function and viral load in liver transplant recipients with recurrent chronic HCV infection. *Liver Transpl* 2004; **10**: 52.
29. Abbott KC, Lentine KL, Bucci JR, *et al.* Impact of diabetes and hepatitis after kidney transplantation on patients who are affected by hepatitis C virus. *J Am Soc Nephrol* 2004; **15**: 3166.
30. Torres J, Aguado JM, San Juan R, *et al.* Hepatitis C virus, an important risk factor for tuberculosis in immunocompromised: experience with kidney transplantation. *Transpl Int* 2008; **21**: 873.
31. Maylin S, Martinot-Peignoux M, Moucari R, *et al.* Eradication of hepatitis C virus in patients successfully treated for chronic hepatitis C. *Gastroenterology* 2008; **135**: 821.
32. Bernardin F, Tobler L, Walsh I, Williams JD, Busch M, Delwart E. Clearance of hepatitis C virus RNA from the peripheral blood mononuclear cells of blood donors who spontaneously or therapeutically control their plasma viremia. *Hepatology* 2008; **47**: 1446.
33. MacParland SA, Pham TN, Guy CS, Michalak TI. Hepatitis C virus persisting after clinically apparent sustained virological response to antiviral therapy retains infectivity *in vitro*. *Hepatology* 2009; **49**: 1431.
34. Alric L, Kamar N, Bonnet D, *et al.* Comparison of liver stiffness, fibrotest and liver biopsy for assessment of liver fibrosis in kidney-transplant patients with chronic viral hepatitis. *Transpl Int* 2009; **22**: 568.
35. Formann E, Steindl-Munda P, Hofer H, *et al.* Long-term follow-up of chronic hepatitis C patients with sustained virological response to various forms of interferon-based anti-viral therapy. *Aliment Pharmacol Ther* 2006; **23**: 507.
36. McHutchison JG, Poynard T, Esteban-Mur R, *et al.* Hepatic HCV RNA before and after treatment with interferon alone or combined with ribavirin. *Hepatology* 2002; **35**: 688.

© 2009 The Authors

37. Wiegand J, Jackel E, Cornberg M, *et al.* Long-term follow-up after successful interferon therapy of acute hepatitis C. *Hepatology* 2004; **40**: 98.
38. George SL, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729.
39. Thomopoulos K, Giannakoulas NC, Tsamandas AC, *et al.* Recurrence of HCV infection in a sustained responder after chemotherapy for non-Hodgkin's lymphoma: successful retreatment. *Am J Med Sci* 2008; **336**: 73.
40. Lin A, Thadareddy A, Goldstein MJ, Lake-Bakaar G. Immune suppression leading to hepatitis C virus re-emergence after sustained virological response. *J Med Virol* 2008; **80**: 1720.
41. Izopet J, Sandres-Saune K, Kamar N, *et al.* Incidence of HCV infection in French hemodialysis units: a prospective study. *J Med Virol* 2005; **77**: 70.

© 2009 The Authors