

Hepatitis C virus RNA in anti-HCV positive hemodialyzed patients: Significance and therapeutic implications

STANISLAS POL, RAFFAELLA ROMEO, BRIGITTE ZINS, FRANÇOISE DRISS, BERNARD LEBKIRI, FRANÇOISE CARNOT, PIERRE BERTHELOT, and CHRISTIAN BRÉCHOT

Unité d'Hépatologie, Banque du sang and Laboratoire d'Anatomo-Pathologie, Hôpital Laënnec, Service d'Hémodialyse de l'Hôpital Necker, de la Clinique de l'Alma and du Parc, Monceau; Laboratoire Hybridotest, Institut Pasteur; INSERM U-99 and U-370, Paris, France

Hepatitis C virus RNA in anti-HCV positive hemodialyzed patients: Significance and therapeutic implications. About 25% of French hemodialysis patients have antibodies against the hepatitis C virus (HCV), which may reflect either past or active HCV infection. It is important to evaluate the significance of these antibodies, as most hemodialysis patients are candidates for kidney transplantation and have normal transaminase activities despite biopsy-proven chronic hepatitis. We prospectively assayed HCV viremia with the nested polymerase chain reaction in 61 patients on maintenance hemodialysis who had anti-HCV antibodies detectable in second generation tests (ELISA2 or RIBA2). HCV RNA was repeatedly detected in the serum of 52 (85.2%) patients. Liver biopsy, which was performed in 17 cases, revealed chronic hepatitis in 16 cases (including 2 of cirrhosis) and steatosis in one. Hypertransaminasemia was observed in only 31.3% and 30.8% of patients with chronic hepatitis and HCV viremia, respectively. Anti-HCV antibodies are frequently associated with HCV viremia, resulting usually in chronic hepatitis, although hypertransaminasemia is uncommon. HCV viremia reflects both post-transfusional and community-acquired HCV infection. These findings suggest a need for liver biopsy and antiviral treatment before kidney transplantation. The isolation of anti-HCV positive subjects in the dialysis setting should be evaluated to reduce patient-to-patient transmission of HCV.

Hemodialyzed patients and kidney transplant recipients are often multiply transfused and are thus frequently infected by hepatotropic viruses, mainly hepatitis B virus (HBV) and hepatitis C virus (HCV) [1-4]. HCV is a single-stranded RNA virus which is a major cause of post-transfusional and sporadic "non-A,non-B" hepatitis. A high prevalence of anti-HCV antibodies has been reported in hemodialysis patients in various geographic areas (5 to 85% worldwide, 25% in France) [5] relative to blood donors (around 0.7%) [6]. This high frequency of HCV infection is related to the number of blood transfusions and the duration of dialysis [7, 8].

The significance of anti-HCV antibodies is not clear; they may reflect either past, resolved infection or active infection with viral replication. There are few data on HCV viremia in hemodialysis patients. Two recent studies reported a high frequency of HCV viremia in hemodialysis patients [9] as well

as kidney transplant recipients [10], but there was no information about liver histology. The significance of anti-HCV antibodies is important for several reasons: (1) some hemodialysis patients are candidates for kidney transplantation, which is usually associated with a worsening of liver lesions [11]; (2) at least 50% of these patients may have normal serum transaminase activity despite biopsy-proven chronic hepatitis [12]; (3) viremic patients could be the source of nosocomial transmission of HCV in hemodialysis units; (4) α -interferon treatment may be of value before kidney transplantation.

The aim of this prospective study was to evaluate the significance of anti-HCV antibodies in hemodialysis patients.

Methods

Inclusion criteria were chronic dialysis and the presence of anti-HCV antibodies.

Patients

In May 1992, we conducted a prospective study of 61 anti-HCV positive patients who were undergoing dialysis in three separate centers. There were 31 men and 30 women, with a mean age of 56 years. The mean dialysis period was 9 ± 6 years, and all but six patients who had never been given blood products had received more than 5 units of blood.

Methods

Serological assays. HBsAg, antiHBs and antiHBc antibodies were detected using an enzyme-linked-immunosorbent assay (Institut Pasteur, Paris, France), according to the manufacturer's instructions.

Serum antibodies to HCV were detected with the second-generation HCV enzyme-linked immunosorbent assay (ELISA2) or recombinant immunoblot assay (RIBA2) from Ortho Diagnostic, according to the manufacturer's instructions. These assays detect antibodies directed against five recombinant HCV antigens: 5-1-1 polypeptide, produced in *E. coli* clones of HCV as a fusion protein with human superoxide dismutase (SOD); the SOD fusion protein C-100-3 produced by selected yeast clones; SOD protein itself, which is used as a control; and C-33-C and C-22-3, which are respectively non-structural and structural proteins.

HCV RNA detection by means of the polymerase chain reaction. HCV RNA purification. RNA was extracted from 150

μ l of serum, reverse transcribed into cDNA and amplified by PCR as previously described [13, 14]. Briefly, the serum was diluted to a final volume of 800 μ l in 0.05 M Tris-HCl (pH 7.5), 0.01 M EDTA, 0.1 M NaCl and 0.5% sodium dodecyl sulphate, digested with 500 μ g/ml proteinase K, extracted with phenol/chloroform, and precipitated with ethanol.

Preparation and amplification of HCV cDNA. To improve the accuracy of the nested PCR assay, HCV cDNA was amplified using a modified nested PCR method [13] and primers located in the 5' non-coding region [15]. Amplified products were stained with ethidium bromide and hybridized with a radiolabeled oligonucleotide probe within the amplified sequence. Drastic contamination-control measures were applied, including physical separation of the laboratories involved in the pre- and post-PCR steps, and inclusion of negative controls throughout (lysis buffer and normal serum). The results were only taken into account when all these controls were negative. Each sample was tested in at least two different runs. In addition, the results were recently validated by the screening of positive and negative reference samples from an international panel (a gift of Dr. J. Reesink, blood bank, Amsterdam).

Primers. Outer primers were antisense (SR1) 5'-TGCACG-GTCTACGAGACCTC-3', corresponding to position (-)2/(-)20 of the prototype HCV cDNA nucleotide sequence, and (SF1) 5'-GCCATGGGGTTAGTATGAGT-3', corresponding to position (-)259/(-)240. Inner primers were antisense (SR2) 5'-GGGCACTCGCAAGCACCCTAT-3' (position (-)25/(-)45) and (SF2) 5'-GTGCAGCCTCCAGGACCCCC-3', corresponding to position (-)235/(-)217. The probe was 5'-ACACCG-GAATTGCCAGGACGACCGGGTCTTCTTG-3' (position (-)176/(-)138).

Serum transaminases activities. Serum aspartate aminotransferase and alanine aminotransferase activities were measured in every case by using the serum from the same blood sample as that used for the serological assays. Enzyme activities were assayed using a fast centrifugal analyzer (Cobas Bio Roche). Hypertransaminasemia was arbitrarily defined as a value over 1.5-fold the upper limit of normal.

Histopathological analysis

Liver biopsy samples were embedded in paraffin. Sections were stained with hematoxylin and eosin and Perls stain. Chronic liver disease was classified as chronic hepatitis or cirrhosis according to criteria which have been recently emphasized [16]. Indeed, chronic persistent hepatitis and chronic active hepatitis are not distinct diseases, and one could change to the other.

Results

All the 61 anti-HCV-positive HBs antigen-negative patients had anti-HCV antibodies detectable in the second-generation serological assays, and HCV viremia was repeatedly detected in the serum of 52 (85.2%) patients (Table 1, Fig. 1).

A liver biopsy was performed in 17 patients who were candidates for kidney replacement. Chronic hepatitis was diagnosed in 16 cases (cirrhosis in two), and steatosis in one patient. Only five of the 16 patients (31.3%) with biopsy-proven chronic hepatitis had increased transaminase activities.

Fourteen of the 15 patients who had HCV viremia had chronic liver disease, including one patient with active cirrhosis

Table 1. Results of HCV RNA detection in the 61 anti-HCV-positive hemodialyzed patients, according to the results of transaminases activities and liver biopsy

	HCV RNA positivity N (%)	HCV RNA negativity N (%)
Overall population	52 (85.2)	9 (14.8)
Hypertransaminasemia	16 (30.8)	2 (22.2)
Patients who underwent liver biopsy	15 (88.2)	2 (11.8)
Biopsy-proven chronic hepatitis	14 ^a (93.3)	2 (100)

^a including 2 with cirrhosis

and one with cirrhosis but no histological features of active disease (Table 1). Hypertransaminasemia was observed in four of these 15 viremic patients (26.7%). Two patients without detectable HCV viremia who underwent liver biopsy also had chronic active hepatitis; one of these two patients with chronic hepatitis and no detectable HCV viremia had increased transaminase activities.

In the 52 PCR-HCV-positive subjects, hypertransaminasemia was present in only 16 (30.8%), while of the 9 PCR-HCV-negative patients only one (11.1%) had hypertransaminasemia.

Six patients had never received blood transfusions; they all had HCV viremia and two (33.3%) had hypertransaminasemia. Forty-six of the 54 patients (85.2%) who had received blood transfusion had HCV viremia, and 11 (20.0%) had hypertransaminasemia.

Finally, abdominal ultrasonography was normal in all but the two patients with cirrhosis who had signs of liver dysmorphism and portal hypertension.

Discussion

Our results show a high frequency of HCV RNA in the serum of French anti-HCV-positive patients on hemodialysis, indicating the existence of on-going viral replication, as recently described in the Far East [9]. In addition, HCV viremia usually results in chronic active hepatitis in hemodialyzed patients, thus evidencing the need of therapeutic antiviral trials.

This high frequency of HCV viremia (85.2%) in hemodialysis patients who were not selected on the basis of liver function tests suggests that the procedure is associated with an increased risk of progression to chronic infection. Indeed, in the general population, around 50% of HCV infection results in chronic hepatitis [17]. An increased risk of chronic HCV infection in hemodialyzed patients, as in the case of HBV infection, may reflect an immunologic deficit related either to uremia or to hemodialysis [18].

It is noteworthy that hypertransaminasemia was observed in only 31% of the patients who had HCV viremia and in less than 30% of those who had biopsy-proven chronic hepatitis. It should be noted that this does not preclude previous intermittent increase of transaminase activities in some of our patients. These results are similar to those reported recently in symptom-free blood donors with serum anti-HCV antibodies [19]. Sixteen of 23 such patients had histological evidence of chronic hepatitis. All 16 patients had HCV viremia, including nine with

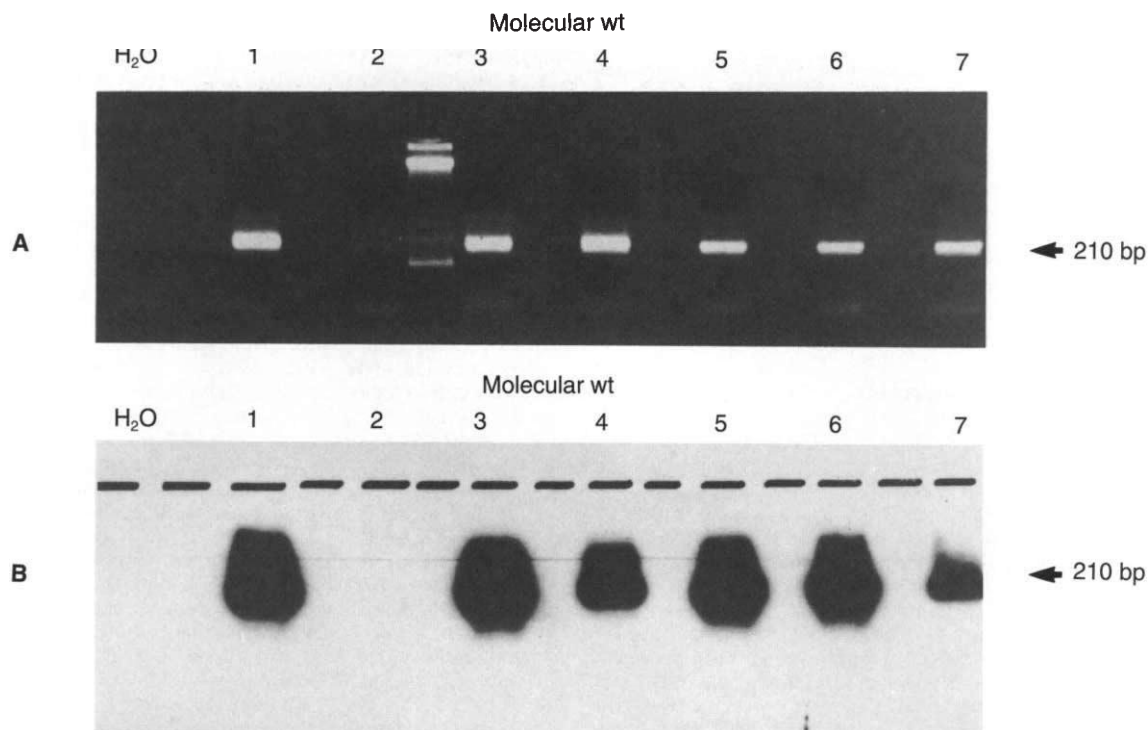


Fig. 1. Detection of HCV-RNA in hemodialyzed patients by polymerase chain reaction. **A.** Ethidium bromide staining. **B.** Autoradiography after ³²P hybridization. Abbreviations are: H₂O, polymerase chain reaction with mix alone; MW, molecular weight marker. Lanes 1, 3, 4, 5, 6 and 7 correspond to positive serum samples for HCV viremia; Lane 2 corresponds to a negative serum for HCV viremia.

normal alanine aminotransferase values, whereas seven HCV-RNA negative patients had a normal liver histology. These results in the overall population as well as in hemodialyzed patients show that serum HCV viremia is a sensitive and specific marker of liver disease in anti-HCV-positive subjects, whatever the transaminase values.

A characteristic course of chronic hepatitis in hemodialyzed patients has been reported, with no marked increase in serum transaminases, mild liver-cell necrosis and inflammatory infiltration [12]. Nevertheless, this course, although remarkably asymptomatic, may lead to a progressive aggravation of histological lesions and cirrhosis may ensue, as was the case in two of our patients. The risk of worsening of the histological index in hemodialyzed subjects raises the difficult problem of liver biopsy. We consider that all anti-HCV-positive hemodialysis patients who are candidates for kidney transplantation should undergo liver biopsy (transcutaneous or transvenous route, or "plugged" biopsy). Indeed, laboratory tests are of little value for determining whether chronic liver disease is present and chronic hepatitis is usually associated with an histological worsening of the liver disease in kidney transplantation, whatever the HBsAg status [11]. Also, alpha-interferon therapy might probably increase the risk of acute vascular rejection in kidney recipients [20]. Thus, an antiviral treatment should probably be given while these patients are waiting for a matched allograft.

HCV may not be the only virus implicated in chronic active hepatitis of HBs antigen-negative anti-HCV-positive hemodialyzed patients. Biopsy-proven chronic hepatitis was indeed observed in two anti-HCV-positive patients who had no evi-

dence of HCV viremia and who were protected against HBV and had no detectable HBV DNA in their serum. This suggests either HCV viremia fluctuated or that a non-A, non-B, non-C virus is responsible for the chronic hepatitis.

Anti-HCV antibodies (and thus HCV viremia) usually correlated with the duration of the dialysis period and the number of blood transfusions [7, 8], which suggests two routes of HCV transmission: community-acquired and post-transfusional infection. The increasing use of recombinant erythropoietin and the better efficiency of blood donor screening has probably resulted in a dramatic decrease in post-transfusional HCV hepatitis in these patients [21]. By contrast, the risk of nosocomial or "community-acquired" HCV transmission persists. This possibility is suggested by: (1) the six anti-HCV-positive subjects from the present study who had never received blood transfusions yet had HCV viremia; (2) outbreaks of non-A, non-B hepatitis previously observed in the dialysis setting, and unrelated to blood transfusions [22]. This hypothesis will now require further evidence such as tests for the detection of HCV on the dialysis machines (water filters, ultrafiltrates, etc.). As with HBV, contamination of environmental surfaces (swabs, clamps, gloves) and the use of the same dialysis shift could be important risk factors. For these reasons, it should be interesting to evaluate the efficacy of the segregation of anti-HCV-positive patients and the complete sterilization of the dialysis machines in limiting patient-to-patient transmission.

In conclusion, anti-HCV antibodies are generally associated with HCV viremia in hemodialysis patients and reflect post-transfusional as well as community-acquired HCV infection. Although hypertransaminasemia is infrequent, HCV viremia

usually results in a chronic hepatitis. These findings call for a systematic sterilization of the dialysis machines, a segregation of anti-HCV-positive patients in the dialysis setting, together with a liver biopsy and antiviral treatment before kidney transplantation.

Acknowledgments

The authors thank Ms. D. Jourdat and Mr. D. Young for their technical assistance in the manuscript preparation.

Reprint requests to Stanislas Pol, M.D., Ph.D., Service d'Hépatologie, Hôpital Necker, 149 rue de Sèvres, 75747 Paris Cedex 15, France.

References

- DEBURE A, DEGOS F, POL S, DEGOTT C, CARNOT F, LUGASSY C, LACOMBE M, KREIS H: Liver diseases and hepatic complications in renal transplant patients. *Adv Nephrol* 17:375-400, 1988
- ELISAF M, TSIANOS E, MAVRIDIS A, DARDAMANIS M, PAPPAS M, SIAMOPOULOS KC: Antibodies against hepatitis C virus (anti-HCV) in haemodialysis patients: Association with hepatitis B serological markers. *Nephrol Dial Transplant* 6:476-479, 1991
- MONDELLI MU, SMEDILE V, PIAZZA V, VILLA G, BARBIERI C, GATTARELLO G, MANCINI F, RAIMONDO G: Abnormal alanine aminotransferase activity reflects exposure to hepatitis C virus in haemodialysis patients. *Nephrol Dial Transplant* 6:480-483, 1991
- SCHLIPKÖTER U, ROGGENDORF M, ERNST G, RASSHOFER R, DEINHARDT F, WEISE A, GLADZIWA U, LUZ N: Hepatitis C virus antibodies in haemodialysis patients. (letter) *Lancet* 335:1409, 1992
- POL S, LEGENDRE C, SALTIEL C, CARNOT F, BRÉCHOT C, BERTHELOT P, MATTLINGER B, KREIS H: Hepatitis C virus in kidney recipients. Epidemiology and impact on renal transplantation. *J Hepatol* 15:202-206, 1992
- JANOT C, COUROUCÉ AM, MANIEZ M: Antibodies to hepatitis C virus in French blood donors. (letter) *Lancet* 2:294-297, 1989
- KALLINOWSKI B, THEILMANN L, GMELIN K, RAMBAUSEK M, MOHRING M, KOMMERELL B: Incidence and prevalence of antibodies to hepatitis C virus in kidney transplant patients. *J Hepatol* 12:404-405, 1991
- MULLER GY, ZABALETA ME, ARMINIO A, COLMENARES CJ, CAPRILES FI, BIANCO NE, MACHADO IV: Risk factors for dialysis-associated hepatitis C in Venezuela. *Kidney Int* 41:1055-1058, 1992
- CHAN TM, LOK ASF, CHENG IKP, CHAN RT: Prevalence of hepatitis C virus infection in hemodialysis patients: A longitudinal study comparing the results of RNA and antibody assays. *Hepatology* 17:5-8, 1993
- CHAN TM, LOK ASF, CHENG IKP, CHAN RT: A prospective study of hepatitis C virus infection among renal transplant recipients. *Gastroenterology* 104:862-868, 1993
- POL S, DEBURE A, DEGOTT C, CARNOT F, LEGENDRE C, BRÉCHOT C, KREIS H: Chronic hepatitis in kidney allograft recipients. *Lancet* 335:878-880, 1990
- DEGOTT C, DEGOS F, JUNGERS P, NARET C, COUROUCÉ AM, POTET F, CROSNIER J: Relationship between liver histopathological changes and HBsAg in 111 patients treated by long-term hemodialysis. *Liver* 3:377-384, 1983
- FERAY C, SAMUEL D, THIERS V, GIGOU M, PICHON F, BISMUTH A, REYNES M, MAISONNEUVE P, BRECHOT C: Reinfection of liver graft by hepatitis C virus (HCV) after liver transplantation. *J Clin Invest* 89:1361-1365, 1992
- NALPAS B, THIERS V, POL S, DRISS F, THÉPOT V, BERTHELOT P, BRÉCHOT C: Hepatitis C viremia and anti-HCV antibodies in alcoholics. *J Hepatol* 14:381-384, 1992
- OKAMOTO H, OKADA S, SUGIYAMA M, YOTSUMOTO S, TANAKA T, YOSHIZAWADA H, TSUDA F, MIYAKAWA Y, MAYUMI M: The 5' terminal sequence of hepatitis C virus genome. *Jpn J Exp Med* 60:167-177, 1990
- SCHEUER PJ: Classification of chronic viral hepatitis: A need for reassessment. *J Hepatol* 13:372-374, 1991
- ALTER HJ, PURCELL R, SHIH JW, MELPOLDER JC, HOUGHTON M, CHOO Q-L, KUO G: Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 321:1494-1500, 1989
- KRUZ P, KÖHLER H, MEUER SC, HÜTTEROTH T, MEYER ZUM BÜSCHENFELDE K-H: Impaired cellular immune responses in chronic renal failure. Evidence for a T cell defect. *Kidney Int* 29:1209-1214, 1986
- ALBERTI A, MORSICA G, CHEMELLO L, CAVALLETTO D, NOVENTA F, PONTISSO P, RUOL A: Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. *Lancet* 340:697-698, 1992
- DAVIS GL: Interferon treatment of viral hepatitis in immunocompromised patients. *Semin Liver Dis* 9:267-272, 1989
- DONAHUE JG, MUNOZ A, NESS PM, BROWN DE, YAWN DH, MCALLISTER HA, REITZ BA, NELSON KE: The declining risk of post-transfusion hepatitis C virus infection. *N Engl J Med* 327:369-373, 1992
- MARCHESI D, ARICI C, POLETTI E, MINGARDI G, MINOLA E, MECCA G: Outbreak of Non-A, Non-B hepatitis in centre haemodialysis patients: A retrospective analysis. *Nephrol Dial Transplant* 3:795-799, 1988