HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis

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HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis. Patients undergoing chronic hemodialysis are at risk for infection with hepatitis B virus (HBV) and hepatitis C virus (HCV). As peripheral blood mononuclear cells (PMNC) are known to be susceptible to infection of both HBV and HCV, assessment of viral genomes in those cells could uncover occult infections not detected by serologic methods or virus determination in serum. We investigated all 67 patients undergoing chronic hemodialysis at a single dialysis unit by PCR for the presence of HBV or HCV genomes in serum as well as in PMNC. None of the 67 patients was HBsAg positive or showed HBV-DNA in serum, but in 5 patients HBV-DNA in PMNC was detected as the only marker of HBV-infection; those patients were also anti-HBc negative. In 9 patients HCV-RNA was positive in serum; in 5 of those patients it was also found in PMNC. Three of these infected patients were negative for anti-HCV. One other patient had no anti-HCV or HCV-RNA in serum, but was positive for HCV-RNA in PMNC. Thus, in 6 patients (8.9%) undergoing chronic hemodialysis we found evidence of infection with HBV or HCV by detecting viral genomes in PMNC without the presence of viremia, antigenemia or specific viral antibodies in serum. The detection of viral genomes in PMNC could be useful in the positive identification of additional potentially infectious patients.

Patients undergoing chronic intermittent hemodialysis carry a variable risk for acquiring infections with hepatitis B (HBV) and hepatitis C (HCV) virus [1]. With HBV infection still present but less prevalent in that group (5.6%) [1], the growing impact of HCV infection has been documented in several surveys showing seroprevalence rates from 8% to 55% [2, 3]. Several variables such as duration of hemodialysis [4], past history of blood transfusion or number of blood units transfused [5] were associated with infection.

The currently available tools for detecting infection with HBV or HCV by testing for virus-specific antibodies, circulating viral antigens, or blood-borne viral genomes (HBV-DNA or HCV-RNA) are powerful methods, but have inherent limitations either due to the given sensitivity of the tests, low viremia, or due to host factors such as a compromised immune system leading to impaired antibody response. Besides being detectable in serum, both HBV [6] and, more recently, HCV [7, 8] have been shown to infect peripheral blood mononuclear cells

(PMNC), as well. Infected PMNC have been implicated as a potentially infectious viral reservoir [9] where HBV and HCV might undergo extrahepatic replication. Even in the absence of HBV-DNA [6] or HCV-RNA in serum [10] hepatitis virus genomes can be present in PMNC. Thus, testing for HBV or HCV viral genomes in PMNC might enhance the chances of detecting viral infection.

We investigated the prevalence of HBV and HCV infection in patients undergoing chronic hemodialysis by testing for viral antigens (HBsAg, HBeAg), viral antibodies (anti-HBs, anti-HBc, anti-HBe, anti-HCV), as well as the presence of viral genomes (HBV-DNA and HCV-RNA) in both serum and PMNC. We report that a considerable percentage of patients has evidence of infection with HBV or HCV in PMNC in the absence of viremia, antigenemia or specific viral antibodies.

Methods

Patients

All 67 patients (35 male, 32 female, mean 55 ± 16 years, range 22 to 83 years) undergoing chronic hemodialysis at dialysis unit I of the Universitätsklinik für Innere Medizin III, University of Vienna, were investigated. Renal disorders resulting in end-stage kidney disease were chronic glomerular nephritis (19 patients), diabetic nephropathy (8 patients), analgesic nephropathy (6 patients), interstitial nephritis (9 patients), and miscellaneous causes (8 patients). In 17 patients the etiology of end-stage renal disease was not established. Mean duration of chronic hemodialysis was 44 ± 34 months, the mean number of transfused blood units was 3.9 ± 3.7 (range: 0 to 25). In regular intervals (every 3 months) infection with hepatitis virus B or C was checked by serologic methods. Serological determination of HBV markers (HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe; Abbott) and of HCV markers (3rd generation; Abbott Laboratories) were performed by ELISA method using commercially available kits. New patients entering the chronic hemodialysis program were routinely assessed for the presence of anti-HBs; 52 patients negative for anti-HBs were vaccinated with double dose of a recombinant hepatitis B vaccine (Engerix B; Smith, Kline, Beecham) given i.m. three times in monthly intervals. This vaccination regime resulted in the seroconversion to anti-HBs in 32 patients (62%), whereas 20 patients (38%) were non-responders to vaccination. Laboratory data of the patients included in this study are given in Table 1.

Table 1. Liver function tests of patients (*N* = 67) undergoing chronic hemodialysis

	All patients	Patients with[a]	Patients without[b]	*P*[c]
		Evidence for presence of viral genomes		
Number of patients	67	15	52	
AST *U/liter*	9 ± 9	13 ± 12	8 ± 7	0.008
ALT *U/liter*	9 ± 8	14 ± 9	8 ± 7	0.045
γGT *U/liter*	48 ± 98	99 ± 150	33 ± 74	0.022
AP *U/liter*	152 ± 113	227 ± 148	130 ± 91	0.003
CHE *kU/liter*	3.9 ± 1.1	3.2 ± 1.2	4.0 ± 1.1	0.012
bilirubin *mg/dl*	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	NS
albumin *g/liter*	42 ± 3.8	41 ± 5.3	42 ± 3.4	NS

[a] positive for HBV-DNA or HCV-RNA in serum and/or PMNC
[b] negative for HBV-DNA or HCV-RNA in serum and PMNC
[c] *P* values of *t*-test between patients with and without evidence for presence of viral genomes

Controls

Serum and PMNC of 16 HBsAg+ patients with chronic hepatitis B, of 15 patients with chronic hepatitis C, and of 21 healthy control persons were also analyzed.

Processing of blood samples

Serum samples and heparinized peripheral venous blood was collected at the same time from each patient using the vacutainer system (Becton Dickinson). All sample handling was performed under sterile conditions in a laminar air flow system in a dedicated room separated from the area where PCR reactions were set up. The recommended precautions to avoid contamination by PCR product carryover and false-positive results were followed [11]. Peripheral blood mononuclear cells (PMNC) were separated by buoyant density gradient centrifugation using Ficoll-Hypaque (Pharmacia). The mononuclear cell fraction was aspirated and washed six times with 30 ml of sterile 0.9% saline. This extensive washing step has been shown in our laboratory to remove serum-associated viral contamination from the PMNC fraction; PCR from an aliquot of the last washing fluid was performed in each patient and gave consistently negative results. Purified and washed PMNC were counted, 2 to 5 × 10⁶ PMNC were lysed in 500 μl 4 M guanidium isothiocyanate/1% 2-mercaptoethanol, and stored at −70°C together with serum samples and an aliquot of the last washing fluid.

Preparation of DNA samples

(a) From serum samples and last washing fluid of PMNC. One hundred microliters of serum or washing fluid were incubated overnight at 37°C with 100 μl double-destilled H_2O and 200 μl 2 × lysis buffer [0.1 M Na_2EDTA, 0.1 M Hepes, 0.4 M NaCl, 4% SDS; pH 7.2, proteinase K (2 mg/ml), yeast tRNA (50 μg/ml)]. The solution was phenol/chloroform extracted and DNA was precipitated with isopropanol, dried and resuspended in 100 μl H_2O.

(b) From PMNC. Two to 5 × 10⁶ PMNC were lysed with 500 μl of 4 M guanidium isothiocyanat containing 1% mercaptoethanol. The lysed cells were dialyzed in separate containers overnight in 1000 ml of TE buffer (10 mM Tris pH 8.0, 1 mM EDTA) at 4°C and incubated for one hour at 55°C with 10 μl of 10% SDS and 50 μl of proteinase K (100 mg/ml). The solution was extracted two times

with phenol/chloroform, total DNA was precipitated with isopropanol, and resuspended in 100 μl H_2O. Total DNA content was quantitated spectrophotometrically at 260 nm.

Oligonucleotide primers for HBV-PCR

A set of nested oligonucleotide primers specific for HBV was used according to Kaneko, Feinstone and Miller [12]. As outer primers the oligonucleotides 1763, 5'-GCT-TTG-GGG-CAT-GGA-CAT-TGA-CCC-GTA-TAA-3', and 2032R, 5'-CTG-ACT-ACT-AAT-TCC-CTG-GAT-GCT-GGG-TCT-3', and as inner primers the oligonucleotides 1778E, 5'-GAC-GAA-TTC-CAT-TGA-CCC-GTA-TAA-AGA-ATT-3', and 2017RB, 5'-ATG-GGA-TCC-CTG-GAT-GCT-GGG-TCT-TCC-AAA-3', were used. The specificity of the amplified PCR product was assessed by hybridization with the internal oligonucleotide HBVOLI1, 5'-GTT-ACT-CTC-GTT-TTT-GCC-TTC-TGA-CTT-CTT-3'.

PCR amplification of HBV-DNA

(a) From serum and washing fluid. DNA extracted from 1 μl of serum or the last washing fluid from PMNC was amplified in a 100 μl reaction volume containing 1 U of *Taq* polymerase (Perkin Elmer Cetus), 11 nmol dNTP, 10 pmol of each of the primers 1763 and 2032R in PCR buffer (10 mM Tris pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$). The polymerase chain reaction was performed in a thermocycler 9600 (Perkin Elmer Cetus) in 200 μl MicroAmpReaction tubes (Perkin Elmer Cetus) and optimized according to the following protocol: 5 minutes 95°C, 30 cycles of 15 seconds at 94°C, 15 seconds at 55°C, 30 seconds at 72°C, followed by 7 minutes 72°C. After completion of the first PCR 1 μl of the reaction volume was transferred to a second PCR tube containing primers 1778E and 2017RB and run for 30 cycles as described above. We were routinely able to detect 1 ag of cloned HBV-DNA corresponding to 1 to 3 HBV particles per μl serum.

(b) From PMNC. One microgram of total DNA isolated from PMNC was amplified in a nested PCR reaction using the same PCR primers, PCR buffer and dNTP concentration as described above, but concentrations of primers (100 pmol) and of *Taq* polymerase (2.5 U) were optimized for the higher total DNA content. Cycling conditions consisted of 5 minutes at 95°C, and 30 cycles of 1 minute at 94°C, 1 minute at 55°C, 1 minute at 72°C, followed by an extension step for 7 minutes at 72°C. Sensitivity was similar as compared to PCR from serum: 1 μg of total cellular DNA (roughly corresponding to 1 × 10⁵ PMNC) spiked with 1 ag of cloned HBV-DNA gave a positive result.

Preparation of RNA samples

(a) From serum and the last washing fluid of PMNC. 50 μl of serum or last washing fluid was added to 200 μl of phosphate-buffered saline and precipitated with 250 μl 16% polyethylene glycol by incubation at 4°C overnight [13]. After precipitation the tubes were spun down in a microfuge for 20 minutes at 4°C and the supernatant was discarded. The pellet was totally dissolved in 500 μl denaturing solution (4.4 M guanidium thiocyanate, 25 mM sodium citrate pH 7.0, 0.5% sarcosyl, 1% 2-mercaptoethanol). After extraction with phenol/chloroform/isoamylalcohol 50 μl of tRNA (1 mg/ml) was added and nucleic acids were precipitated with absolute ethanol at −20°C overnight. After a further spun in the microfuge for 20 minutes at 4°C the pellet was washed with 70% ethanol and centrifuged. Ethanol was drawn off and the tubes were placed in a biological safety cabinet until the pellet was

dry. Thereafter the pellet was resuspended in 100 μl of double-destilled water containing 40 U/μl RNAsin (Promega).

(b) From PMNC. Two to 5 × 10^6 PMNC were lysed with 1 ml lysis buffer [10 mM EDTA (pH 8.0), 0.5% SDS] and 1 ml of 0.1 mM sodium acetate (pH 5.2) and 10 mM EDTA (pH 8.0). This solution was extracted twice with phenol/chloroform. Nucleic acids were precipitated twice with ice cold 1 M Tris-HCl (pH 8.0), 5 M NaCl, and ethanol. The pellet was redissolved in 200 μl H_2O containing 7 U RNAsin and stored at −70°C.

Oligonucleotide primers for HCV-PCR

A set of nested oligonucleotide primers specific for the 5'-untranslated region of HCV was used according to Hsu et al [14]. As outer primers the oligonucleotides F1, 5'-GGC-GAC-ACT-CCA-CCA-TAG-ATC-3', and R1, 5'-GGT-GCA-CGG-TCT-ACG-AGA-CCT-3', and as inner primers the oligonucleotides F3, 5'-CGT-TGA-GGA-ACT-ACT-GTC-TTC-3', and R3, 5'-CCC-TAT-CAG-GCA-GTA-CCA-CAA-3', were used.

PCR amplification of HCV-RNA

(a) From serum and washing fluid. PCR was performed in a volume of 100 μl containing RNA extracted from 1 μl serum, 1 U *Taq* polymerase, 1 U reverse transcriptase (Promega), 16 U RNAsin, 40 pmol of each of the outer primers F1 and R1, 4 nmol dNTP in PCR buffer. The cycling protocol, optimized for the thermocycler 9600 (Perkin-Elmer) and the 200 μl MicroAmpReaction tubes, was as follows: reverse transcription 30 minutes at 42°C, 5 minutes at 95°C; 25 cycles of 15 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 72°C; extension step of 7 minutes at 72°C. After completion of the first PCR 1 μl of the reaction volume was transferred to a second PCR tube containing primers F3 and R3, 1 U *Taq* polymerase, 4 nmol dNTP in PCR buffer. The cycling protocol was 4 minutes at 95°C; 25 cycles of 15 seconds at 95°C, 30 seconds at 55°C, 30 seconds at 72°C; and an extension step of 7 minutes at 72°C. We were able to detect RNA corresponding to 8 to 10 HCV particles/μl serum.

(b) From PMNC. Cycling conditions, primer concentrations (50 pmol), and concentration of *Taq* polymerase (2.5 U) were optimized for the high content of nucleic acids. The cycling protocol was identical to that used for serum except denaturing, annealing, and extension times were prolonged to 1 minute each. We were able to detect the lowest positive dilution of a HCV-RNA positive standard serum when added to 1 × 10^5 uninfected PMNC, indicating that sensitivity of HCV-PCR from PMNC was in the same range as from serum.

Analysis of amplified DNA

The amplified DNA product was analyzed on a 2% agarose (Pharmacia) gel under UV-light after staining with ethidium bromide. The expected amplicon of HBV-PCR was 256 bp, that of HCV-PCR 259 bp long. In every PCR run two reagent controls, two positive, and two negative samples were included. All samples were run in duplicate from the beginning of the extraction procedure until gel analysis and congruent result only were accepted.

Results

HBV-infection

HBV serology. None of our 67 patients undergoing chronic hemodialysis was HBsAg+. 17 patients had signs of past HBV

Table 2. Characteristics of patients with HBV-DNA or HCV-RNA in PMNC

Viral genome in PMNC	Serological status	PCR in serum
Patients with HBV-DNA in PMNC *N* = 5 (7.5%)		
3	HBsAg− anti-HBs+ anti-HBc+	HBV-DNA−
2	HBsAg− antiHBs− anti-HBc−	HBV-DNA−
0	HBsAg− anti-HBs+ anti-HBc+	HBV-DNA−
0	HBsAg− antiHBs− anti-HBc+	HBV-DNA−
Patients without HBV-DNA in PMNC *N* = 62 (92.5%)		
29	HBsAg− anti-HBs+ anti-HBc−	HBV-DNA−
16	HBsAg− anti-HBs− anti-HBc−	HBV-DNA−
15	HBsAg− anti-HBs+ anti-HBc+	HBV-DNA−
2	HBsAg− anti-HBs− anti-HBc+	HBV-DNA−
Patients with HCV-RNA in PMNC *N* = 6 (8.9%)		
3	Anti-HCV+	HCV-RNA+
2	Anti-HCV−	HCV-RNA+
0	Anti-HCV+	HCV-RNA−
1	Anti-HCV−	HCV-RNA−
Patients without HCV-RNA in PMNC *N* = 61 (91.1%)		
4	Anti-HCV+	HCV-RNA+
1	Anti-HCV−	HCV-RNA+
3	Anti-HCV+	HCV-RNA−
53	Anti-HCV−	HCV-RNA−

infection (anti-HBc+), 15 of them were anti-HBs+, two were anti-HBs−. All 52 anti-HBs− patients had been included in a vaccination program: 32 of the vaccinated patients were classsified as responders, whereas 20 were non-responders to vaccination.

HBV-DNA in serum. In none of the patients could HBV-DNA be detected in serum by PCR.

HBV-DNA in PMNC. In 5 out of 67 patients (5.7%) HBV-DNA was detected in PMNC (Table 2). Interestingly, HBV in PMNC was found in 3 of 32 anti-HBs+ anti-HBc− responders to vaccination and in 2 of 18 anti-HBs− and anti-HBc− non-responders to vaccination, but in none of the 17 patients with markers of past HBV infection (anti-HBc+). HBV-DNA was present in PMNC of 13 out of 16 patients with chronic hepatitis B, but was not detectable in PMNC of 21 healthy controls.

HCV-infection

HCV-serology. Ten (15%) of the 67 patients undergoing chronic hemodialysis were positive for anti-HCV.

HCV-RNA in serum. Only 7 of the 10 anti-HCV+ patients had HCV-RNA in serum. However, in 3 (5.3%) of the remaining 57 anti-HCV− patients HCV-RNA was found to be present in serum.

HCV-RNA in PMNC. We found HCV-RNA in PMNC in 6 (8.9%) of 67 patients (Table 2). Three of them were also anti-HCV+ and serum HCV-RNA+, 2 were anti-HCV− but serum HCV-RNA+, whereas 1 patient was both anti-HCV− and serum HCV-RNA−. HCV-RNA could be detected in PMNC of all 15 patients with chronic hepatitis C, but not in PMNC of 21 healthy controls.

Double infection with HBV and HCV. One of our patients had evidence for infection with both HBV and HCV. HBV-DNA was found in PMNC and HCV-RNA in serum, but neither serological markers for HBV (HBsAg−, anti-HBc−, serum HBV-DNA−) nor HCV (anti-HCV−) infection were present.

Clinical correlations

We compared clinical features of patients (N = 15 (22%)) with the presence of viral genomes (HBV-DNA or HCV-RNA in serum and/or PMNC) with those of patients (N = 52; 78%) without evidence for the presence of hepatitis viral genomes (negative for HBV-DNA and HCV-RNA in serum and PMNC). Only 13% of the infected patients undergoing hemodialysis had elevated aminotransferases; the mean values were within the normal range (AST (13 ± 12 U/liter), ALT (14 ± 9 U/liter). Patients with evidence for the presence of hepatitis viral genomes had significantly higher serum levels of markers for cholestasis, such as alkaline phosphatase and γGT, and lower levels of pseudocholinesterase than patients without evidence for the presence of hepatitis viral genomes (Table 1). No other laboratory differences were found between these two groups. Neither mean duration of hemodialysis (58 ± 54 months vs. 41 ± 30 months) nor number of blood units transfused (6 ± 4 vs. 5 ± 6) was different in these groups. No difference in laboratory and clinical parameters was found in patients with infection of PMNC only as compared to patients with viral infection detected in serum.

Discussion

In the present paper we describe the prevalence of HBV and HCV genomes in PMNC of patients undergoing chronic hemodialysis and present evidence that in 8.9% of patients infection with those viruses is detectable in PMNC only, without concomitant presence of viremia, antigenemia or specific antibodies in serum. Thus, testing of PMNC for infection with hepatitis virus B and C is useful in the positive identification of potentially infectious patients.

HBV [6] and HCV [7, 8, 10] are able to infect PMNC, as shown in both patients with [6, 10] and without [15, 16] liver disease as evidenced by elevated serum aminotransferases. HBV and HCV genomes are transcribed [7, 17] and replicate [17] in PMNC, and are thought to constitute an important viral reservoir which possibly accounts for recurrence of hepatitis virus infection in certain clinical situations such as liver transplantation [9, 18].

HBV and HCV viral sequences have been found in a high percentage in PMNC in patients with circulating serum HBV-DNA [19] and HCV-RNA [16], respectively. In addition, both the HBV virus genome [15] and HCV-RNA [10] have also been demonstrated in PMNC in the absence of detectable hepatitis virus genomes in serum. Similarly, in hepatitis B infection the presence of HBV-DNA in tissues other than PMNC, such as liver, in the absence of HBV-DNA in serum has not only be demonstrated using sensitive PCR techniques [20], but also by relatively insensitive hybridization assays [6]. These findings are in accor-

dance with results obtained in the patients investigated in this study: we found HBV-DNA in PMNC in five patients and HCV-RNA in PMNC in one patient without concomitant presence of viral genomes in serum. Thus, testing for HBV-DNA or HCV-RNA in PMNC in addition to serum is a more sensitive approach than serum testing alone.

Furthermore, HBV-DNA and HCV-RNA have been detected in PMNC in patients without any serologic markers of HBV [15, 21] or HCV infection [10]. This was also seen in our patients undergoing chronic hemodialysis as HBV-DNA was found in PMNC of 2 of 67 (2.9%) patients without any serologic markers for HBV infection. Similarly, in 5.3% of patients without anti-HCV PMNC were positive for HCV-RNA. Our findings are further illustration of the well known fact that hepatitis viral genomes can be present in the absence of serological markers of infection.

In addition, HBV-DNA can be found in serum even in the absence of any serologic markers of HBV infection [21]. This was not the case in our patients. However, in hepatitis C the detection of HCV-RNA in sera of anti-HCV negative patients has frequently been reported in hemodialysis patients [2, 22]; this was the case in one of our patients. An impaired immune response in patients with uremia [23] might be the reason for the absence of specific antibodies.

In three anti-HBc negative patients HBV-DNA was found in PMNC despite the presence of anti-HBs which were the result of previous HBV vaccinations; it is tempting to speculate that PMNC are a site of long-lasting latent HBV infection which might persist even beyond the time of gradual loss of naturally acquired anti-HBs and anti-HBc. The anti-HBs in these patients might be an anamnestic immune response to vaccination given to individuals with unrecognized persistent infection.

False positive results are always of concern when using PCR techniques. We think, however, that strict adherence to precautions as outlined by Kwok and Higuchi [11], the use of a closed system for blood drawing (vacutainer), and the addition of negative controls in every PCR run makes false-positive reactions due to contaminations unlikely. Furthermore, HBV-DNA and HCV-RNA in PMNC do not seem to be a carryover of virus particles from serum as: (i) positive results were seen in patients with PCR-negative serum; (ii) the last washing fluid of PMNC was always PCR negative; and (iii) and actual transcription of HBV-DNA [17] or HCV-RNA [7, 10, 16] as opposed to positivity due to mere adherence of virus particles to the cell membrane of PMNC has been convincingly demonstrated.

Most of our patients with evidence of HBV or HCV infection had normal serum aminotransferase levels. Only 2 out of 15 infected patients had ALT levels above normal. Although patients with evidence for presence of viral genomes had higher serum levels of ALT as compared to those without evidence for viral infection, the absolute ALT values were well within normal range. Patients with evidence for hepatitis viral infection had increased markers of cholestasis (γGT, alkaline phosphatase), as described for this patient group [24]. No difference could be found in laboratory parameters in patients with infection of PMNC only, compared to patients with hepatitis virus present in serum. These data are in agreement with reports for HBV [25] and HCV [22] infection in hemodialysis patients in which ALT levels had no predictive value for hepatitis virus infection due to the frequent finding of normal ALT values.

Patients undergoing chronic hemodialysis are at an increased risk to acquire HBV and HCV infection [1]. Several factors such as duration of hemodialysis [4], number of blood units transfused [5], and non-separation of infected from non-infected patients [26] have been associated with infection. However, even in dialysis units with low prevalence of HBV and HCV infection and strict adherence to guidelines for avoiding transmission of blood-borne infectious agents [27], the infection rate with hepatitis viruses is still higher than in the general population [28]. Patients with hepatitis virus genomes in PMNC but without serologic evidence of infection or viral genomes in serum might constitute a hidden source of infection and contribute to "sporadic" infection in dialysis units [29]. Similarly, after renal transplantation patients are still at risk to acquire hepatitis virus infection [30] even without a continuing need for hemodialysis or blood transfusion. Unrecognized chronic infection with HBV or HCV, possibly reactivated by immunosuppression after transplantation, might be the cause for reappearance of serologically documented infections in these patients; by searching for HBV and HCV in PMNC occult infections with these viruses might be uncovered.

In conclusion, a small, but significant proportion of hemodialysis patients have evidence for infection with HBV and HCV in PMNC despite the absence of viral genomes, viral antigens, or specific viral antibodies in serum. Thus, surveillance performed by serology and PCR from serum may underestimate the true rate of infection with hepatitis viruses. These data underscore the need for adequate and continuing prophylactic measures, even in a population of hemodialysis patients who are negative for serologic viral markers or serum hepatitis genomes, to limit the spread of hepatitis virus infection within dialysis units.

References

1. RAINE AEG, MARGREITER R, BRUNNER FP, EHRICH JHH, GEERLINGS W, LANDAIS P, LOIRAT C, MALLICK NP, SELWOOD NH, TUFVESON G, VALDERRABANO F: Report on management of renal failure in Europe, XXII, 1991. *Nephrol Dial Transplant* 7(Suppl 2):7–35, 1992
2. BUKH J, WANTZIN P, KROGSGAARD, KNUDSEN F, PUCELL RH, MILLER RH, THE COPENHAGEN DIALYSIS HCV STUDY GROUP: High prevalence of hepatitis C virus (HCV) RNA in dialysis patients: Failure of commercially available antibody tests to identify a significant number of patients with HCV. *J Infect Dis* 168:1343–1348, 1993
3. ZELDIS JB, DEPNER TA, KURAMOTO IK, GISH RG, HOLLAND PV: The prevalence of hepatitis C virus antibodies among hemodialysis patients. *Ann Intern Med* 112:958–960, 1990
4. HARDY NM, SANDRONI S, DANIELSON S, WILSON WJ: Antibody to hepatitis C virus increases with time on hemodialysis. *Clin Nephrol* 38:44–48, 1992
5. HUANG C-S, HO M-S, YANG C-S, LEE C-L, TAN CA: Hepatitis C markers in hemodialysis patients. *J Clin Microbiol* 31:1764–1769, 1993
6. NOONAN CA, YOFFE B, MANSELL PW, MELNICK JL, HOLLINGER FB: Extrachromosomal sequences of hepatitis B virus DNA in peripheral blood mononuclear cells of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci USA* 82:5698–5702, 1986
7. ZIGNEGO AL, MACCHIA D, MONTI M: Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 15:382–386, 1992
8. BOUFFARD P, HAYASHI PH, ACEVEDO R, LEVY N, ZELDIS JB: Hepatitis C virus is detected in a monocyte/macrophage subpopulation of peripheral blood mononuclear cells of infected patients. *J Infect Dis* 166:1276–1280, 1992
9. FÉRAY C, SAMUEL D, THIERS V: Reinfection of liver graft by hepatitis C virus after liver transplantation. *J Clin Invest* 89:1361–1365, 1992
10. MURATORI L, GIOSTRA F, CATALETA M, FRANCESCONI R, BALLARDINI G, CASSANI F, LENZI M, BIANCHI FB: Testing for hepatitis C virus sequences in peripheral blood mononuclear cells of patients with chronic hepatitis C in the absence of serum hepatitis virus RNA. *Liver* 14:124–128, 1994
11. KWOK S, HIGUCHI R: Avoiding false positives with PCR. *Nature (London)* 339:237–238, 1989
12. KANEKO S, FEINSTONE SM, MILLER RH: Rapid and sensitive method for the detection of serum hepatitis B virus DNA using the polymerase chain reaction technique. *J Clin Microbiol* 27:1930–1933, 1989
13. CRISTIANO K, DI BISCEGLIE AM, HOOFNAGLE JH, FEINSTONE SM: Hepatitis C viral RNA in serum of patients with chronic non-A, non-B hepatitis: Detection by the polymerase chain reaction using multiple primer sets. *Hepatology* 14:51–55, 1991
14. HSU HH, WRIGHT TL, LUBA D, MARTIN M, FEINSTONE SM, GARCIA G, GREENBERG HB: Failure to detect hepatitis C virus genome in human secretions with the polymerase chain reaction. *Hepatology* 14:763–767, 1991
15. HADCHOUEL M, PASQUINELLI C, FOURNIER JG, HUGON JG, SCOTTO J, BERNARD O, BRÉCHOT C: Detection of mononuclear cells expressing hepatitis B virus in peripheral blood from HBsAg positive and negative patients by in situ hybridisation. *J Med Virol* 24:27–32, 1988
16. WILLEMS M, PEERLINCK K, MOSHAGE H, DELEU I, VAN DEN EYDEN C, VERMYLEN J, YAP SH: Hepatitis C virus-RNAs in plasma and in peripheral blood mononuclear cells of hemophiliacs with chronic hepatitis C: Evidence for viral replication in peripheral blood mononuclear cells. *J Med Virol* 42:272–278, 1994
17. REPP R, MANCE A, KELLER C, RHIEL S, GERLICH WH, LAMPERT F: Detection of transcriptionally active hepatitis B virus DNA in peripheral mononuclear blood cells after infection during immunosuppressive chemotherapy using the polymerase chain reaction. *Arch Virol* 4(Suppl):50–53, 1992
18. FÉRAY C, ZIGNEGO AL, SAMUEL D, BISMUTH A, REYNES M, TIOLLAIS P, BISMUTH H, BRÉCHOT C: Persistent hepatitis B virus infection of mononuclear blood cells without concomitant liver infection. *Transplantation* 49:1155–1158, 1990
19. PASQUINELLI C, MELEGARI M, VILLA E, SCAGLIONI PP, SEIDENARI M, MONGIARDO N, DE RIENZO B, MANETI F: Hepatitis B virus infection of peripheral blood mononuclear cells is common in acute and chronic hepatitis. *J Med Virol* 31:135–140, 1990
20. KUHNS M, MCNAMARA A, MASON A, CAMPBELL C, PERRILLO R: Serum and liver hepatitis B virus DNA in chronic hepatitis B after sustained loss of surface antigen. *Gastroenterology* 103:1649–1656, 1992
21. PREISLER-ADAMS S, SCHLAYER H-J, PETERS T, HETTLER F, GEROK W, RASENACK J: Sequence analysis of hepatitis B virus DNA in immunologically negative infection. *Arch Virol* 133:385–396, 1993
22. SILINI E, BONO F, CERINO A, PIAZZA V, SOLCIA E, MONDELLI MU: Virologic features of hepatitis C virus infection in hemodialysis patients. *J Clin Microbiol* 31:2913–2917, 1993
23. REVILLARD JP: Immunologic alterations in chronic renal insufficiency. *Adv Nephrol* 8:365–382, 1979
24. SAKAMOTO N, ENOMOTA N, MARUMO F, SATO C: Prevalence of hepatitis C virus infection among long-term hemodialysis patients: Detection of hepatitis C virus RNA in plasma. *J Med Virol* 39:11–15, 1993
25. WARE AJ, GORDER NL, GURIAN LE, DOUGLAS C, SHOREY JW, PARKER T: Value of screening markers of hepatitis in dialysis units. *Hepatology* 3:513–518, 1983
26. SNYDMAN DR, BERGMAN D, BRYAN JA: Hemodialysis associated with hepatitis in the United States. *J Infect Dis* 135:687–691, 1977
27. CENTERS FOR DISEASE CONTROL, ATLANTA: Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *JAMA* 260:462–465, 1988
28. YAMAGUCHI K, NISHIMURA Y, FUKUOKA N, MACHIDA J, UEDA S, KUSUMOTO Y, FUTAMI G, ISHII T, TAKATSUKI K: Hepatitis C virus antibodies in haemodialysis patients. *Lancet* 335:1409–1410, 1990
29. SHUSTERMAN N, SINGER I: Infectious hepatitis in dialysis patients. *Am J Kidney Dis* 9:447–455, 1987
30. CHAN T-M, LOK ASF, CHENG IKP, CHAN RT: A prospective study of hepatitis C virus infection among renal transplant recipients. *Gastroenterology* 104:862–868, 1993