Different kinetics of HBV and HCV during haemodialysis and absence of seronegative viral hepatitis in patients with end-stage renal disease

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Abstract

Background. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are common in haemodialysis units. Moreover, some studies reported seronegative cases of viral hepatitis. We and others have previously shown an HCV RNA decline during haemodialysis; however, limited data on HBV viraemia during haemodialysis are available.

Methods. A total of 142 haemodialysis patients participated in this study, 11 were anti-HCV positive and 7 were HBsAg positive. HCV RNA and HBV DNA were determined in all
patients irrespective of hepatitis serology. HBV DNA, HCV RNA, HBsAg and HCV core antigen (HCVcoreAg) were quantified repeatedly in anti-HCV- and HBsAg-positive patients before and after haemodialysis.

**Results.** No case of seronegative viral hepatitis could be identified. HCV RNA was detected in 9 of the 11 anti-HCV-positive patients, while HBV DNA tested positive in all 7 HBsAg-positive patients. A decrease of HCVcoreAg was observed during four dialysis sessions in 8/9 patients (−24.4 ± 22.7%, P < 0.001) paralleled by HCV RNA decline in most individuals (−10.1 ± 48.6%, P = 0.22). In contrast, HBV DNA and HBsAg declined only in 1/7 patients during all four independent measurements. The remaining six patients showed heterogeneous patterns of HBV DNA and HBsAg before and after haemodialysis without a significant change in mean HBV DNA and HBsAg levels (+14 ± 60.6% and −0.2 ± 25.3%, P > 0.05, respectively). HCVcoreAg correlated strongly with HCV RNA (r = 0.937; P < 0.001, n = 72), while there was no correlation between HBV DNA and HBsAg (r = −0.234; P = 0.131, n = 43).

**Conclusions.** Seronegative viral hepatitis is rare in German maintenance haemodialysis patients. HCV RNA and HCVcoreAg decline during haemodialysis indicating a potential beneficial effect of haemodialysis during antiviral therapy of hepatitis C, which does not apply to HBV infection.

**Keywords:** haemodialysis; HBsAg; HCVcoreAg; occult hepatitis B; seronegative hepatitis C

**Introduction**

Infections with different types of viral hepatitis are common in haemodialysis centres. The prevalence of a positive anti-hepatitis C virus (HCV) test among dialysis patients was 5.4% in a large prospective multicenter trial in Germany [1] and up to 9.8% in the US dialysis population [2]. However, in southern Europe and Asia, the prevalence may be even higher with rates up to 22.9% [3]. In contrast, hepatitis B virus (HBV) infection prevalence among dialysis population ranges between 2.1 and 4.6% in western countries [4]. Screening for newly acquired hepatitis C should be performed by monthly ALT determination and biannually testing of hepatitis C antibodies [5]. For prevention of hepatitis B infection, vaccination against HBV is recommended [6, 7], although the non-responder rate after vaccination is significantly higher than in a non-dialysis population [8]. In case of vaccination failure, regular screening of HBsAg should be performed [6]. However, haemodialysis patients show an impaired immune response and antibody screening might not be sufficient for early detection of a viral infection due to a lack or delay in antibody production. Thus, in some cases, HCV RNA or HBV DNA testing might be required to detect seronegative infected patients [9–11]. Seronegative patients are more likely to display a low level of viral replication [12], which does not rule out infectivity. Occult hepatitis B—the detection of HBV DNA in the serum in the absence of HBsAg—has also been described in patients on maintenance haemodialysis [11] and could also be a mechanism of hepatitis B transmission. HCV RNA and HBV DNA testing improved significantly over the last decade with lower limits of detection of 5–20 IU/mL HCV RNA or HBV DNA with the latest available assays [13–17]. Recently, a new fully automated HCV core antigen (HCVcoreAg) test has become available on the same platform as quantitative hepatitis B surface antigen (HBsAg) [18], which can be used to confirm ongoing viral replication [19, 20].

It has been shown previously that haemodialysis per se can lead to reduction of HCV RNA and HCVcoreAg [21–23], which might explain the better response to antiviral treatment in most patients; however, the pattern did not seem universal in all patients [21]. A viral load reduction has also been reported for HBV DNA during haemodialysis sessions [24, 25] and viral load was found to be relatively low in HBV-infected dialysis patients [26, 27].

This study aimed to investigate two aspects: (i) the prevalence of occult hepatitis B and HCV RNA detection in the absence of HCV antibodies in a German outpatient dialysis unit and (ii) the reproducibility of viral kinetics of HCV RNA and HCVcoreAg as well as HBV DNA and HBsAg in individual patients with chronic hepatitis C or hepatitis B on maintenance haemodialysis, respectively, during different haemodialysis sessions.

**Materials and methods**

**Patients**

All patients under stable maintenance haemodialysis therapy in a large single haemodialysis centre in Hannover (Germany) were asked to participate in the study (n = 170). There were no exclusion criteria except for denial of informed consent. A total of 142 patients on maintenance haemodialysis consented for participation in this study and were screened for HCV RNA and HBV DNA, irrespective of hepatitis serology. All screening samples were taken prior to dialysis session before administration of anticoagulant. Those patients positive for anti-HCV (n = 11, one patient was also HBsAg positive, but repeatedly HCV RNA negative) or HBsAg (n = 7) were tested for viral antigens and nucleic acids before and after haemodialysis at four independent time points. Two HBsAg-positive subjects could be tested only once. Anti-HCV-positive patients were evaluated quantitatively for HCV RNA and HCVcoreAg. HBsAg-positive patients were evaluated using quantitative HBsAg and HBV DNA assays. In all patients, a complete blood cell count was performed before and after haemodialysis and the body weight was documented to calculate the loss of fluid during haemodialysis. Serological hepatitis markers and clinical chemistry (anti-HBs, HBsAg, anti-HCV, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, platelets) were performed routinely and documented on a half-year interval basis. None of the patients with viral hepatitis C or B received antiviral treatment in the six months prior to the study entry except for Patient no. 142.

All patients signed an informed consent and the study protocol was approved by the local ethic review board.

**Quantitative virological markers**

Plasma was collected before dialysis in all patients and before and after dialysis when studying viral kinetics on haemodialysis. Samples were stored at −20°C and thawed only for testing.

**HCV RNA and HBV DNA quantification.** HCV RNA and HBV DNA were quantified using the commercially available assays for the COBAS TaqMan platform according to the manufacturer’s instruction (Roche Diagnostics, Mannheim, Germany). The lower limit of detection for HCV RNA as well as HBV DNA was 20 IU/mL. This assay uses an internal standard and would indicate a substantial inhibition by e.g. heparin, which did not occur during any of the polymerase chain reactions (PCRs).

**HCVcoreAg and HBsAg quantification.** HCVcoreAg and HBsAg were quantified using commercially available assays running on the Architect
Haemodialysis

Patients underwent dialysis every other day for at least 4 h in a threetimes-a-week shift (either Monday/Wednesday/Friday or Tuesday/Thursday/Saturday). The blood samples of all patients were taken on Mondays or Tuesdays, after the long weekend. The duration of the respective dialysis sessions in the kinetic studies varied between 4.00 and 7.25 h (for patient with nocturnal haemodialysis). The haemodialysis membranes used were either Nipro-170HGA (cellulose acetate; Serumwerk, Bemburg, Germany) or Nipro-PES-190DH (polyether sulfone; Serumwerk). All patients infected either with hepatitis C or B, who participated in the second part of the study, were dialysed via arteriovenous fistula except for Patient no. 141 who was temporarily dialysed via a central jugular vein catheter (Shaldon catheter). In this patient, the blood sample was taken after the blocking agent (citrate 4%) had been removed and at least 10 mL of whole blood has been previously aspirated via the catheter.

Correction of viral markers considering the fluid loss during dialysis

The markers of viral replication were measured in plasma in the current study. When water is extracted during haemodialysis, the plasma gets concentrated and all content in the plasma shows higher concentrations reflected in haematomcit increases. Smaller molecules like sodium could easily float between plasma and cellular and extracellular compartments. For the purpose of the viral load, we assume that viral particles do not easily flow between compartments, and especially, there is no evidence for significant virus uptake in organs other than the liver. The amount of plasma is lower after haemodialysis. This means that if the same amount of virus in the circulation remained unchanged, one would expect an increase in viral load per millilitre. In other words, the same viral load before and after haemodialysis would in fact indicate a decline during haemodialysis. To correct for fluid loss/plasma concentration, we used the following calculation:

Relative viral load or antigen = 
\[
\begin{align*}
\text{viral load or antigen post-dialysis} \\
\text{viral load/antigen pre-dialysis}
\end{align*}
\]

Corrected relative viral load or antigen = 
\[
\begin{align*}
\text{Relative viral load or antigen} \\
\text{Hct pre-dialysis} \\
\text{Hct post-dialysis}
\end{align*}
\]

Statistics

The significance of the correlation was calculated using two-tailed Pearson’s correlation with SPSS 11.0. The difference of the mean values was calculated using the t-test, paired t-test and one-way analysis of variance, when appropriate. A P-value < 0.05 was considered significant.

Results

Initial screening of dialysis centre

A total of 142 individuals participated in the study. All patients were screened for HBV DNA and HCV RNA in plasma. HCV RNA and HBV DNA were detected in nine and seven patients, respectively. All HCV RNA positive subjects were already known to be anti-HCV positive. Similarly, all HBV DNA positive patients tested HBsAg positive before. Importantly, no case of seronegative hepatitis C or hepatitis B was identified. Results of screening for HCV RNA and HBV DNA are shown in Table 1.

Kinetics of HCV RNA and HCV coreAg during haemodialysis

All nine HCV RNA-positive patients were studied four times for HCV RNA and HCV coreAg before and after haemodialysis. In line with our previous finding [21], HCV coreAg and HCV RNA showed a strong correlation in absolute values (r = 0.937; P < 0.001, n = 72).

A decrease of HCV coreAg during each independent determination before and after haemodialysis was observed in eight of nine patients (mean decrease 24.4 ± 22.7%, P < 0.001, or P = 0.014 paired r-test), which was paralleled by an overall decline in serum HCV RNA (mean decrease 10.1 ± 48.6%, P = 0.22). Only three of nine patients (no. 54, no. 139 and no. 141) showed a decrease of HCV RNA during every haemodialysis, another patient (no. 86) declined with HCV RNA during three of four repeated determinations. Two patients (no. 44 and no. 90) showed a heterogeneous pattern of HCV RNA (decline in two of four measurements), whereas another patient (no. 94) showed an increase of HCV RNA during three of four determinations. Interestingly, in Patient no. 106, HCV RNA levels increased during every dialysis session which was paralleled by an increase of HCV coreAg during three of four dialysis sessions. The absolute HCV coreAg and HCV RNA levels and relative changes of every patient are shown in Figures 1a and 2a.

We further analysed factors that might be associated with a decline/increase of HCV coreAg and HCV RNA during haemodialysis. There was no difference in the mean HCV coreAg and HCV RNA decrease between patients that underwent haemodialysis with the two different dialysis membranes. Furthermore, no association between duration of dialysis and decrease of HCV coreAg/HCV RNA was observed. Markers of liver inflammation, such as ALT levels, as well as age and sex were likewise not associated with a decrease of HCV coreAg/HCV RNA.

Kinetics of HBV DNA and HBsAg during haemodialysis

Of the seven HBV DNA-positive patients, five were available for repeated determinations of HBV DNA and HBsAg before and after haemodialysis. In contrast to HCV RNA and HCV coreAg, we observed a relative HBV DNA increase after haemodialysis (mean increase 14 ± 60.6%, P > 0.05) and on average, no change of HBsAg levels (0.2 ± 25.3%, P > 0.05). Moreover, there was no correlation between HBV DNA and HBsAg (r = -0.234; P = 0.131, n = 43). When viral load change was analysed individually, we saw different patterns of HBV DNA and HBsAg kinetics before and after haemodialysis (see Figure 1b). One patient (no. 142) showed a decrease after haemodialysis for HBV DNA and HBsAg during each of the four determinations. Another patient (no. 4) presented a decrease of HBV DNA and HBsAg three of four times. Interestingly, during the remaining haemodialysis session, HBV DNA and HBsAg increased in parallel. Patient no. 29 showed three of three times a parallel change of HBV DNA and HBsAg, but only one of three times a decrease of HBV DNA and HBsAg in parallel and two of three times an increase of HBV DNA and HBsAg. The remaining two patients with serial determinations (no. 71 and no. 87) revealed heterogeneous patterns of HBV DNA and HBsAg with a decrease and/or increase in half of the determinations. The patients no. 63 and no. 78 were only available for one determination of HBV DNA and HBsAg before and
Discussion

A prolonged time to anti-HCV seroconversion has been reported in patients on maintenance haemodialysis [31] as well as seronegative hepatitis C [32, 33]. Occult hepatitis B has been described especially in anti-HBe-positive patients [34]. In the current study, we screened all patients on maintenance haemodialysis with ultrasensitive HCV RNA and HBV DNA PCR assays irrespective of results from hepatitis serology. Importantly, using this approach, we did not find a single case of seronegative hepatitis C or occult hepatitis B and thus, HCV RNA or HBV DNA was detected only in renal patients known to be anti-HCV or HBsAg positive. The absence of seronegative hepatitis C and occult hepatitis B in our cohort might be explained by different factors. Firstly, with regards to HCV and HBV Germany is a low endemic area with <1% of people being infected with either of these viruses [35]. Secondly, the haemodialysis unit where the study was performed adheres to the strict hygienic regulations on haemodialysis in Germany including separation of infected patients and regularly testing for viral hepatitis [36]. However, these regulations, especially the strict separation of dialysis machines demonstrated no efficacy in preventing the nosocomial transmission of hepatitis C as the majority of cases occurred via contaminated gloves, surfaces or medical equipment [5]. In line with our finding, an earlier study demonstrated also the absence of new HCV or GB virus C infection in a prospective study of haemodialysis patients [1]. The same study also did not find a case

Table 1. Baseline characteristics and results of screening for HCV RNA and HBV DNA

<table>
<thead>
<tr>
<th></th>
<th>Negative for HBsAg and Anti-HCV</th>
<th>Anti-HCV positive</th>
<th>HBsAg positive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>125/142 (88%)</td>
<td>10/142 (7%)</td>
<td>7/142 (5%)</td>
<td>n.a.</td>
</tr>
<tr>
<td>Age in years</td>
<td>70.5 ± 14.2</td>
<td>63.8 ± 15.4</td>
<td>59.3 ± 21.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gender: male/female</td>
<td>68/57 (54%)</td>
<td>9/1 (90%)</td>
<td>4/3 (57%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI in kg/m²</td>
<td>25.6 ± 5.4</td>
<td>22.7 ± 4.0</td>
<td>21.6 ± 2.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT in U/L</td>
<td>19.8 ± 9.0 (4)</td>
<td>29.7 ± 30.8² (1)</td>
<td>21.7 ± 9.5 (0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(no. of patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT &gt; ULN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST in U/L</td>
<td>19.3 ± 7.0</td>
<td>23.3 ± 14.1</td>
<td>22.7 ± 9.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Albumin in g/L</td>
<td>41.0 ± 3.9</td>
<td>39.0 ± 3.3</td>
<td>44.4 ± 7.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Platelets 10⁹/L</td>
<td>245 ± 83</td>
<td>175 ± 56b</td>
<td>212 ± 58</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HBV DNA positive (%)</td>
<td>0/125</td>
<td>0/10</td>
<td>7/7</td>
<td>n.a.</td>
</tr>
<tr>
<td>HCV RNA positive (%)</td>
<td>0/125</td>
<td>9/10</td>
<td>0/7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Race Caucasian (%)</td>
<td>124/125 (99%)</td>
<td>10/10 (100%)</td>
<td>7/7 (100%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>124/125 (33%)</td>
<td>5/10 (50%)</td>
<td>2/7 (29%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>History of kidney transplantation (%)</td>
<td>7/125 (6%)</td>
<td>4/10 (40%)</td>
<td>3/7 (43%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time since dialysis started in years</td>
<td>5.4 ± 5.2</td>
<td>11.4 ± 9.7b</td>
<td>9.5 ± 9.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

A total of 142 patients were screened. One patient (no. 29) was HBsAg and anti-HCV positive. As HCV RNA tested repeatedly negative, the patient was listed in the group of HBsAg-positive patients and was considered as HCV recovered. One-way analysis of variance was performed to test for significant differences between the means of the different parameters between the different patient groups. ULN for ALT and AST were 35 and 50 U/L for women and men, respectively. Chi-square test was used to test categorical data for statistical significance. ULN, upper limit of normal.

bSignificant difference between the uninfected group (negative for HBsAg and anti-HCV) and either anti-HCV or HBsAg-positive patients (Dunnett’s t-test).
of seronegative hepatitis C, thus supporting our data [1]. However, in our study, the lower limit of detection for HCV RNA or HBV DNA was 20 IU/mL, whereas an assay with a limit of detection of 250 IU/mL was applied in that earlier study [1].

To further exclude the possibility of seronegative persistent HCV infection, one can consider to apply even more sensitive approaches such as studying HCV RNA in PBMC or liver biopsies [37]. Similarly, HBV DNA can be detected in livers of seronegative individuals [38]. However, the clinical relevance of detection of extremely low levels of HCV RNA or HBV DNA can be questioned in the context of dialysis as it would be extremely unlikely that this residual viraemia represents a reasonable reservoir for viral transmission.

Although there was a significant difference between mean ALT levels between non-infected and infected patients in the current study, only one of the infected patients had an ALT level above the upper limit of normal. This confirms previous data showing lower ALT levels in patients with chronic renal failure [39] and emphasizes the importance of screening for viral hepatitis using serological and molecular assays irrespective of ALT levels.

In the second part of the study, we studied viral kinetics of hepatitis C and B during dialysis. This study was of interest as declines of viral nucleic acids and viral antigens during dialysis have been reported previously, and may have significant implications both for understanding basic mechanisms of viral replication/turnover as well as for the development of novel therapeutic options for difficult to treat patients. Moreover, no study has yet compared both HCV and HBV kinetics in the same experimental setting. We therefore tested for viral antigens and nucleic acids before and after haemodialysis. Of note, each patient was tested several times allowing comparison not only of interindividual differences but also of intraindividual variabilities. Some previous studies described a mostly universal decline in viral loads [24, 25, 40], which we could not confirm here. However, the findings in this study are concordant to an earlier study of ours, where we used another viral load assay [21]. HCVcoreAg decreased during haemodialysis in almost all patients. HCVcoreAg decline was paralleled in most of those patients by an HCV RNA decline. However, in some patients during selected haemodialysis sessions, HCVcoreAg declined and HCV RNA increased (no. 28, no. 44, no. 86, no. 90 and no. 98).

Fig. 1. Overview of absolute values of markers of viral replication before and after haemodialysis. For renal patients infected with HCV (a) or HBV (b), each pair of graphs shows HCV RNA (left graph) and HCVcoreAg (right graph) or HBV DNA (left graph) and HBsAg (right graph), respectively, before and after haemodialysis. In each single graph on the left the value before haemodialysis and on the right the level of viral replication after haemodialysis is diagrammed. The number above the graphs represents the respective patient number, the small number next to the connecting line indicates the haemodialysis session.
94) but not vice versa. This discordance between the consistent decrease of HCVcoreAg during haemodialysis and the variable evolution of HCV RNA in the same patients, however, cannot be related to the use of heparin, which has been claimed to be a partial inhibitor of the PCR [41]. A possible explanation is rather the difference between the coefficient of variation (%CV) between the HCVcoreAg and the Cobas TaqMan HCV RNA assay. According to the manufacturer’s instruction, the CV of the HCVcoreAg assay is <10% compared to up to 50% for the HCV RNA assay [13].

We used a novel approach to correct the viral markers after dialysis for the extracted plasma by a proposed formula. The use of haematocrit for correction seems to us the most appropriate approach as, for example, the body weight does not reflect the fluid status within the vascular system, which is the compartment of interest of this study. After correction for haematocrit, the observed increase of HCV RNA and HCVcoreAg in Patient no. 106 was reduced for HCV RNA and even lost for HCVcoreAg which supports an additional haematocrit-corrected data analysis. Using the haematocrit corrected values, we saw even more...
pronounced decreases of virological markers after haemodialysis. There are several possible explanations for viral load and HCV core Ag reduction during haemodialysis, for example the absorption of viral particles by the haemodialysis membrane [42], the increase of plasma interferon alfa levels after haemodialysis [43] or the removal of a crucial factor required for viral production or maturation [21].

The results for HBV DNA and HBsAg are more heterogeneous. Two other studies that investigated kinetics of HBV DNA during haemodialysis observed a decrease of HBV DNA after haemodialysis [25, 44]. Only one patient in our study showed a continuous decrease of HBV DNA that was paralleled by HBsAg. Interestingly, this is the only renal patient who had been on antiviral treatment (Tenofovir; Gilead Sciences) during the study period. We also noted an increase of HBV DNA in at least one haemodialysis session in the remaining patients; however, viral loads were rather low, arguing also for a technical issue as %CV were also up to 50% when using the HBV DNA assay [16]. However, also HBsAg kinetics were rather heterogeneous too. Three renal patients (no. 29, no. 71 and no. 87) with pre-dialysis HBsAg levels in the same range between 14,000 and 30,000 IU/mL showed an increase or decrease during haemodialysis, which argues for a different mechanism compared to HCV core Ag. One factor that might contribute to a different effect of haemodialysis could be the size of the viral particle, but the size of both viruses is pretty similar with HCV being ~50 nm in diameter and HBV 42 nm [45] and larger than the expected pore size. Another explanation might be viral production rates and viral half-
life. The production rates are pretty similar with $10^{10}$ to $10^{12}$ virions per day [46, 47]; however, the viral half-life has been suggested to be only 3 h for HCV [47] but 2–3 days for HBV [48]. If one of these factors contributes to the different patterns is currently unknown.

In conclusion, seronegative hepatitis C or occult hepatitis B are rare in German patients with end-stage renal disease. Interestingly, we observed different kinetics of HCV and HBV during haemodialysis with a mostly reproducible decrease of markers of hepatitis C, whereas markers of HBV showed heterogeneous patterns.

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