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Increase of HCV RNA concentration during hemodialysis treatment in patients with chronic hepatitis C

Csilla Putz-Bankuti[a,*], Harald H. Kessler[b], Gernot Schilcher[c], Daniel Schneditz[d], Petra M. Konrad[b], Alexander R. Rosenkranz[c], Rudolf E. Stauber[a]

[a] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[b] Research Unit Molecular Diagnostics, Center of Applied Biomedicine, Medical University of Graz, Graz, Austria
[c] Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[d] Institute of Physiology, Center for Physiological Medicine, Medical University of Graz, Graz, Austria

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ABSTRACT

Background: In hemodialysis (HD) patients, a decrease of serum HCV RNA concentration during HD has been reported.
Objectives: To evaluate the effect of two different extracorporeal blood treatments, HD and hemodiafiltration (HDF), on HCV RNA concentration under standardized conditions.
Study design: Eleven chronic HD patients with chronic hepatitis C (CHC) and thirty-three non-uremic patients with CHC as controls were studied. Blood samples were collected at baseline ($t = 0$ min), 30 min ($t = 30$), and 180 min ($t = 180$) after start of HD or HDF. HCV RNA concentrations were determined by a real-time PCR assay. Values obtained 30 min and 180 min after start of HD or HDF were adjusted according to the ultrafiltration-induced hemoconcentration.
Results: Baseline HCV RNA concentrations were found to be similar in dialysis patients and controls (2.9E + 06 vs. 5.8E + 06 IU/ml). After adjustment for hemoconcentration, no significant differences of HCV RNA concentrations were observed when HD versus HDF treatments and blood samples collected pre versus those collected post membrane were compared. Adjusted HCV RNA concentrations increased by 13% (not significant) at 30 min and by 56% ($p < 0.001$) at 180 min after start of HD or HDF. Inhibitory effects on PCR through heparin and uremic toxins could be excluded.
Conclusions: In contrast to recent publications, a significant increase of serum HCV RNA within 180 min after start of HD or HDF was observed. Changes in serum HCV RNA concentration are independent from HD and HDF procedures, dialysis membrane, heparin concentration, and uremic toxins.

1. Background

Chronic hepatitis C (CHC) is a common infection among chronic hemodialysis (HD) patients with a prevalence of up to 3% in northern and up to 30% in southern Europe and these patients should be routinely monitored for hepatitis C virus (HCV) infection.[1–6] In a small proportion of patients with end-stage renal disease (ESRD), HCV RNA can be detected in serum, while lacking anti-HCV antibodies.[3,6] Interestingly, in ESRD patients with CHC, the infection usually shows a mild progression with liver enzymes within normal range, low grade inflammation, and significantly lower percentages of fibrosis.[7–10]

In HD patients, a decrease of serum HCV RNA concentration during HD (15–78%) was reported.[11–15] It was suggested that the virus may be adsorbed onto the membrane surface or destroyed by the pressure applied to blood dialysis or reverse transcription PCR may be partially inhibited through the use of heparin during HD.[11,12,15,16]

2. Objectives

The aim of this study was to evaluate changes of the serum HCV RNA concentration in patients during HD and hemodiafiltration (HDF). Possible confounding variables such as hemoconcentration (HC), heparin concentration, HCV RNA absorption on the dialysis membrane, and inhibition of PCR due to uremic toxins were investigated. Serum HCV RNA concentrations and levels of liver function parameters were studied and compared to those observed in patients with CHC and normal renal function.

* Corresponding author at: Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 16, 8036 Graz, Austria. Tel.: +43 316 385 81937; fax: +43 316 385 13927.
E-mail address: csilla.putz-bankuti@medunigraz.at (C. Putz-Bankuti).

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Table 1
Baseline characteristics of patients studied and controls.

	Patients ($n = 11$)	Controls ($n = 33$)
Age (years)	53 ± 14	51 ± 11
Sex (female:male)	4:7	12:21
HCV genotype 1 (1a or 1b)	5 (46%)	6 (18%)
HCV subtype 1a	4 (36%)	9 (27%)
HCV subtype 1b	2 (18%)	18 (55%)

3. Study design

3.1. Subjects

This prospective study was conducted at the Division of Nephrology, Medical University of Graz, according to principles of the Declaration of Helsinki. The study protocol and all study procedures were reviewed and approved by the local ethics committee. All participants gave written informed consent.

The study group consisted of 11 patients with CHC and ESRD. Inclusion criteria were detectable serum HCV RNA and ESRD on HD for at least 6 months prior to baseline. The control group consisted of 33 non-uremic patients with CHC, detectable serum HCV RNA for at least 6 months, and normal renal function. The mean age, the female to male ratio, and the distribution of HCV geno-/subtypes were similar in both groups (Table 1). Study participants had never received any anti-HCV therapy.

3.2. HD and HDF procedures

All patients studied ($n = 11$) underwent HD first followed by HDF after 48 h. Treatment conditions including ultrafiltration volume (V_u), treatment duration, ultrafiltration rate, and plasma flow rate were comparable between HD and HDF procedures. Polyflux 17L or 170H (Gambro AB, Lund, Sweden) dialyzers and Gambro (Gambro AB, Lund, Sweden) dialysis machines were used to deliver bicarbonate hemodialysis or on-line hemodiafiltration at a blood flow of 250 ml/min and a dialysate flow of 500 ml/min.

3.3. Collection of blood samples

For quantitation of serum HCV RNA, blood was collected in 9-ml tubes (Greiner Bio-One GmbH, Kremsmünster, Austria). Blood samples were obtained from the peripheral or central-venous access at the beginning of the treatment (baseline, $t = 0$ min) and from both the pre membrane (arterial) and post membrane (venous) line of the extracorporeal circulation at times $t = 30$ and $t = 180$ min. Within 2 h of drawing blood, 9-ml tubes were centrifuged at $1500 \times g$ for 20 min at room temperature. After centrifugation, aliquots were prepared and immediately frozen at −70 °C until tested.

3.4. Quantitation of serum HCV RNA

The COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems, Inc., Branchburg, NJ), which is based on automated sample preparation on the COBAS AmpliPrep instrument followed by real-time PCR and detection on the COBAS TaqMan analyzer, was performed according to the manufacturer's package insert instructions. The lower limit of detection is 15 IU/ml.

The effect of the extracorporeal system on HCV RNA concentration (C, in IU/ml) was expressed by the relative extracorporeal change (R_x) calculated from the post-dialyzer concentration in venous line serum returning to the patient (C_v) and the pre-dialyzer concentration in arterial line serum entering the extracorporeal circulation (C_a):

$$R_x = \frac{C_v}{C_a}. \tag{1}$$

3.5. Adjustment for ultrafiltration-induced changes in plasma volume

HD and HDF are accompanied by significant changes in plasma volume because of concomitant ultrafiltration of excess body fluid. For solutes and components distributed in plasma volume such as HCV RNA, serum concentration is expected to increase because of ultrafiltration-induced HC. Therefore, the relative change in plasma or serum concentrations has to be adjusted to account for the change in distribution volume.

The relative change in concentration measured in the extracorporeal system R_x was corrected for the effect of ultrafiltration by the following relationship

$$R_{x\,\mathrm{corr}} = R_x \frac{Q_p - Q_u}{Q_p}, \tag{2}$$

with Q_p and Q_u referring to plasma flow and ultrafiltration rates (in ml/min), respectively. Plasma flow rate Q_p was determined from blood flow rate (Q_b) and arterial line hematocrit (H, in %) as

$$Q_p = Q_b \left(1 - \frac{H}{100}\right). \tag{3}$$

Ultrafiltration rate was determined from the difference of pre- and post treatment body mass divided by treatment time.

The measure $R_{x\,\mathrm{corr}}$ refers to the immediate extracorporeal effect. A value of $R_{x\,\mathrm{corr}} = 1$ implies that (at that specific time point) the plasma (or serum) component is neither removed from nor delivered to the patient when blood passes the extracorporeal circulation.

The relative change in HCV RNA concentrations in the patient (R_p) was determined as the ratio of arterial serum concentration (C_t) relative to baseline serum concentration (C_0) by the following relationship:

$$R_p = \frac{C_t}{C_0}. \tag{4}$$

With ongoing ultrafiltration plasma volume is likely to change over time so that R_p also has to be corrected for the effects of HC as described elsewhere[17]:

$$R_{p\,\mathrm{corr}} = R_p \frac{H_0}{H_t} \left(\frac{100 - H_t}{100 - H_0}\right), \tag{5}$$

H_0 (in %) refers to the hematocrit at baseline, and H_t (in %) to arterial line hematocrit at times $t = 30$ and 180 min, respectively.

The measure $R_{p\,\mathrm{corr}}$ refers to the global treatment effect. A value of $R_{p\,\mathrm{corr}} = 1$ implies that the treatment as a whole is neutral with regard to the plasma (or serum) component of interest.

3.6. Investigation of potential PCR inhibitors

Serum samples in HD patients contain heparin for anticoagulation during HD treatment. To exclude a partial or complete inhibitory effect on PCR, heparin (Heparin Immuno 1000 I.E./ml – Ebewe Pharma, Unterach, Austria) was added to remainders of serum samples with known HCV RNA level that were left following clinical routine testing. Aliquots containing final heparin concentrations of 0.1, 0.5, 1, 5, and 10 IU/ml were prepared and HCV RNA concentrations were determined.

To exclude a partial or complete inhibitory effect on PCR due to uremic retention toxins, 1 ml of pre-dialysis serum from uremic patients was mixed with 1 ml serum of CHC controls with known

HCV RNA concentration. Four sets of samples were prepared, serum HCV RNA concentrations determined, and results compared.

3.7. Statistical analysis

Statistical analyses were performed using SPSS, version 18.0 for windows. The significance of the correlation between patients studied and controls was calculated using Fisher's exact test and ANOVA with p for trend. The descriptive data are presented as means ± standard deviation (SD). Statistical analysis for the evaluation of HCV concentrations under different conditions (HD and HDF) was performed using the non-parametric Friedman test for three variables and the non-parametric one-sample sign test to assess the changes relative to baseline. Statistical significance was indicated by an alpha level of 0.05.

4. Results

In patients studied (n = 11) and controls (n = 33), mean baseline HCV RNA concentrations were found to be similar (2.9E+06 ± 4.9E+06 vs. 5.8E+06 ± 7.4E+06 IU/ml). In contrast, there was a significant difference (p < 0.001) when liver function and renal retention parameters were compared between patients studied and controls (Table 2).

Treatment parameters such as ultrafiltration volume, treatment duration, ultrafiltrations rate, and plasma flow rate were comparable between HD and HDF procedures (Table 3). When HD and HDF procedures were compared, similar results were found. Forty-eight hours after HD and HDF, HCV RNA concentrations were found to be similar to those at baseline in all patients studied.

Table 2
Clinical and laboratory characteristics of 11 patients studied and 33 controls. Data presented as mean ± standard deviation. ANOVA with p for trend and Fisher's exact test were used.

	Patients	Controls	p-value
HCV RNA (IU/ml × E+06)	2.9 ± 3.5	5.8 ± 7.4	0.14
Bilirubin (mg/dl)	0.35 ± 0.7	0.84 ± 0.4	<0.001
Creatinine (mg/dl)	9.7 ± 3.9	0.9 ± 0.2	<0.001
Urea (mg/dl)	119.7 ± 37.8	25.9 ± 8.2	<0.001
ALAT (IU/l)	38 ± 31	87 ± 53	<0.05
ASAT (IU/l)	31 ± 20	73 ± 74	<0.05
CRP (mg/l)	6.1 ± 4.8	1.3 ± 1.5	<0.001
GGT (IU/l)	73 ± 94	118 ± 158	0.38
Leukocytes (G/l)	6.5 ± 1.9	6.2 ± 2.26	0.73
Hematocrit (%)	36.1 ± 4.1	45.3 ± 13.6	0.28
Protein total (g/dl)	7.6 ± 0.7	7.9 ± 0.4	0.1
Albumin (g/dl)	5.8 ± 0.9	6.46 ± 0.56	<0.05

Table 3
Treatment data for 11 HD treatments followed by 11 HDF treatments in patients studied (n = 11).

	HD ± SD	HDF ± SD
$M_{b,post}$ (kg)	78.6 ± 16.2	76.5 ± 16.1
H_0 (%)	35.8 ± 4.1	35.9 ± 3.8
H_{30} (%)	35.4 ± 3.8	34.9 ± 3.1
H_{180} (%)	36.5 ± 2.7	35.5 ± 2.7
V_u (l)	2.6 ± 1.0	2.1 ± 0.9
t (h)	4.0 ± 0.4	4.1 ± 0.2
Q_u (ml/min)	11.0 ± 4.6	8.4 ± 3.7
Q_{p30} (ml/min)	161.5 ± 9.5	162.8 ± 7.8
Q_{p180} (ml/min)	158.8 ± 6.6	161.3 ± 6.7

Abbreviations: SD, standard deviation; HD, hemodialysis; HDF, hemodiafiltration; $M_{b,post}$, post-treatment body mass; H_0, H_{30}, H_{180}, hematocrit at baseline and at 30 and 180 min, respectively; V_u, ultrafiltration volume; t, duration of treatment; Q_u, ultrafiltration rate; Q_{p30}, Q_{p180}, plasma flow rate at 30 min and 180 min, respectively.

Table 4
HCV RNA concentrations (IU/ml × E+06 IU/ml) and relative changes after correction for hemoconcentration (HC) for 11 HD treatments followed by 11 HDF treatments in patients studied (n = 11).

	HD + HDF	SD	HD	SD	HDF	SD
C_0	2.9***	4.9	2.4	3.5	3.3	6.2
C_{a30}	3.4***	5.9	3.2	5.5	3.7	6.7
C_{a180}	3.6***	5.1	3.9	5.3	3.3	5.2
R_{p30}	1.10	0.24	1.20	0.27	1.07	0.23
R_{p180}	1.59†††	0.99	1.78	1.35	1.39	0.37
$R_{p30corr}$	1.13	0.26	1.15	0.30	1.12	0.24
$R_{p180corr}$	1.56†††	0.89	1.72	1.21	1.41	0.36
R_{x30}	1.34††	0.55	1.45	0.75	1.23	0.23
R_{x180}	1.15†	0.32	1.19	0.30	1.11	0.34
$R_{x30corr}$	1.26	0.52	1.35	0.71	1.17	0.22
$R_{x180corr}$	1.07	0.28	1.1	0.26	1.04	0.31

Abbreviations: SD, standard deviation; HD, hemodialysis; HDF, hemodiafiltration; C_0, baseline serum HCV RNA concentration; C_{a30}, C_{a180}, arterial line serum HCV RNA concentration at 30 and 180 min, respectively; R_{p30}, R_{p180}, change in arterial serum HCV RNA concentration at 30 and 180 min relative to baseline; $R_{p30corr}$, $R_{p180corr}$, change in arterial serum HCV RNA concentration at 30 and 180 min relative to baseline corrected for HC; R_{x30}, R_{x180}, relative extracorporeal change in serum HCV RNA concentration at 30 and 180 min; $R_{x30corr}$, $R_{x180corr}$, relative extracorporeal change in serum HCV RNA concentration at 30 and 180 min corrected for HC.

*** p < 0.001, Friedman test.
† p < 0.05.
†† p < 0.01.
††† p < 0.001, relative to baseline (=1, one-sample sign test).

During HD and HDF, the arterial HCV RNA concentration increased by 10% (not significant) and 59% (p < 0.001 relative to baseline) when measured after 30 and 180 min of dialysis, respectively. This increase was maintained at 13% (not significant) and 56% (p < 0.001 compared to baseline) after adjustment for HC (Table 4; Fig. 1). In the blood passing the dialyzer, the HCV RNA concentration was 34% (p < 0.001) and 15% (p < 0.05) higher at the venous outflow than at the arterial inflow when measured after 30 and 180 min of treatment, respectively. However, this relative increase disappeared when adjusted for HC.

The investigation of a potential effect of PCR inhibitors on the HCV RNA concentration measured revealed lack of inhibition by heparin added to the sample up to a concentration of 1 IU/ml did not have any influence on the value obtained. Similarly, samples containing high concentrations of uremic toxins did not show any inhibitory effect (Table 5).

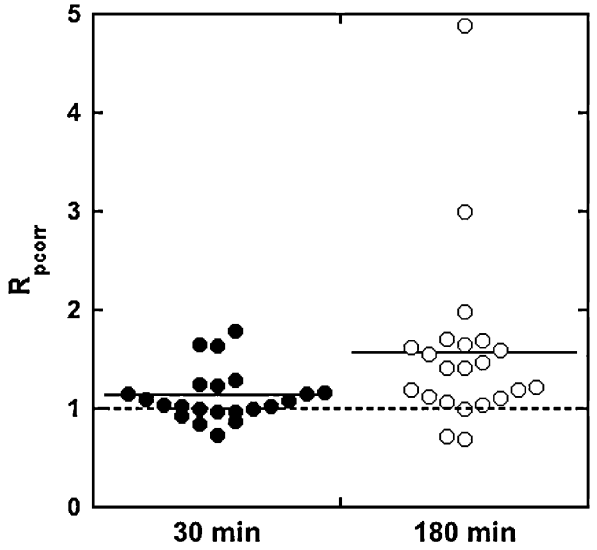

Fig. 1. Relative changes of HCV RNA concentrations in pre membrane (arterial) blood after adjustment for HC at 30 and 180 min of HD and HDF.

Table 5
Investigation of a possible inhibitory effect on PCR due to uremic retention toxins. One ml of pre-dialysis serum from uremic patients was mixed with one ml of serum of CHC controls with known HCV RNA concentration.

	Urea (mg/dl)	Creatinine (mg/dl)	HCV RNA expected (IU/ml × E+06)	HCV RNA measured (IU/ml × E+06)	Log HCV RNA difference
Sample 1	122	9.6	0.5	0.4	0
Sample 2	114	10.3	5.9	4.3	<0.5
Sample 3	123	9.0	7.4	5.6	<0.5
Sample 4	85	3.4	0.4	0.4	0

5. Discussion

In this study, HCV RNA concentrations were studied in a group of 11 patients with ESRD and CHC treated by two different modes of dialysis. Interestingly, HCV RNA concentrations increased rather than decreased during extracorporal therapy, independent of treatment mode HD or HDF. This increase is in contrast to data published recently.[11–15] The HCV RNA increase was not an effect of ultrafiltration-induced HC.

Recent studies reported significantly lower HCV RNA concentrations and lower liver enzymes in individuals with ESRD and CHC compared to those with normal renal function.[7–10] When uremic patients with CHC and chronic dialysis were compared with non-uremic patients with CHC, no differences in HCV RNA concentrations were observed. However, there was a significant difference regarding liver function parameters between patients studied and controls confirming previous studies.

In this study, no significant change of pre versus post membrane HCV RNA concentrations was observed. Furthermore, the HCV concentration increased over time. The significant reduction of the HCV RNA concentration reported in recent studies was explained by several mechanisms including HCV adsorption to the dialysis membrane, destruction of HCV on the membrane, and/or leakage of HCV through the membrane into the dialysate.[11,12,15,16] However, none of those studies analyzed patients under standardized conditions of HD. In this study, the times of blood collection, the duration of HD and HDF procedures, the blood flow, and the dialysate flow were standardized and the pre membrane (arterial) HCV RNA concentration was adjusted according to the substantial change of HC due to the ultrafiltration in the dialyzer. Furthermore, HCV RNA concentrations were determined with an in vitro diagnostics (IVD)/Conformite Europeene (CE)-labeled and FDA cleared fully automated molecular test.

Inhibition has been an obstacle to successful PCR. Common specimen types may contain inhibitors including heparin, heme, and uremic toxins.[18] In particular, heparin has been reported to be one of the most potent PCR inhibitors.[19] The nucleic acid extraction protocol must be able to efficiently remove inhibitors and ensure inhibitor-free nucleic acids for the subsequent amplification reaction.[20] In this study, inhibition of real-time HCV PCR due to heparin could be excluded. Together with real-time PCR, the nucleic extraction protocol used is able to provide reliable HCV concentrations out of samples containing high-level heparin concentrations that were never reached in the patients' blood throughout this study. Furthermore, inhibition due to accumulation of uremic toxins in HD patients was excluded. Additionally, the molecular assay used in this study contains a homologous internal control to exclude false-negative results due to interference from inhibitors. The internal control which was added before the start of the nucleic acid extraction procedure to ensure an accurate control of the entire molecular assay was detected together with all target results.

In conclusion, no significant change of pre versus post membrane HCV RNA concentrations but a significant increase of serum HCV RNA within 180 min after start of HD or HDF was observed in contrast to recent studies. Dialysis procedures, the collection site, heparin concentration, heme, and uremic toxins did not show an effect on the serum HCV RNA concentration. However, the HCV RNA concentration was affected significantly by the duration of the dialysis procedure. Further investigations are required to clarify the relation between changes in HCV RNA concentration and the slow progression of CHC in HD patients.

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Competing interests

None declared.

Ethical approval

The study was approved by the local ethics committee and written informed consent was obtained from all patients.

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