# Measurement of hydroxychloroquine clearance in intermittent hemodialysis

François Seghers[1], Kévin-Alexandre Delongie[2], Pierre Wallemacq[2], Laura Labriola[1,3]

[1]Department of Nephrology Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

[2]Department of Clinical Chemistry, Cliniques universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium

[3]Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, Brussels, Belgium

**Address for correspondence:**

Laura Labriola, M.D.

Department of Nephrology

Cliniques universitaires Saint-Luc

Université catholique de Louvain

Avenue Hippocrate 10

B 1200 Brussels

BELGIUM

Phone: +32 2 7641852

Fax: +32 2 7642836

e-mail: laura.labriola@uclouvain.be

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## Background

Hydroxychloroquine (HCQ) has been used for decades in the treatment of rheumatoid arthritis, systemic lupus erythematosus (SLE) and other inflammatory rheumatic diseases (1). Its mechanisms of action are multiple and include alterations of lysosomal functions, membrane stability and intracellular signaling pathways (2). Very recently there was an intense focus on HCQ since it was proposed as a potential treatment for SARS-CoV-2 infections (COVID-19). Because of its narrow therapeutic index, the use of HCQ raises safety concerns in patients on maintenance dialysis, since there is no data supporting HCQ dose adjustment in CKD stage 4-5. However, HCQ dose adjustment is of great importance in order to reach therapeutic efficacy (disease activity control), and to avoid cardiac and retinal toxicity in acute or chronic overdosing, respectively (3).

HCQ is a small aqua-soluble compound (335 Da), with a good bowel absorption after oral intake and a high bioavailability (0.7-0.8) (2). It is protein-bound at 40% and has a high volume of distribution (800 L/kg). Its blood terminal elimination half-life is extremely long (40–60 days) (4,5). HCQ undergoes metabolism into desethylated metabolites, whose clinical effects seem modest. The rate of renal clearance of unchanged HCQ is 21%, and is even lower for its metabolites (4).

On the basis of its structure and pharmacokinetics similarities with chloroquine, HCQ is considered theoretically as non-dialyzable (1). However, data concerning HCQ pharmacokinetics in hemodialysis (HD) are lacking. Even though one might assume dialysability of a drug according to its characteristics such as molecular weight, volume of distribution and protein binding capacity, confirmative data are needed for its safe use in maintenance HD patients. In this setting, we were concerned about the efficacy of HD in clearing HCQ and its impact on HCQ blood therapeutic levels. Our aim was thus to assess blood

HCQ concentrations, and HCQ dialytic clearance, removal and post-dialysis rebound, in two maintenance HD patients on long-term HCQ treatment for SLE.

## Methods

***Measures:***

Measurements were performed on a GENIUS 90 Therapy System (Fresenius Medical Care, Bad Homburg, Germany) with a Fx Cordiax 600 (1.2 m²) hollow-fiber high-flux polysulfone dialyser (Fresenius Medical Care). Blood and dialysate flows were set at 250 mL/min and 290 mL/min for Patients 1 and 2, respectively. Dialysate composition was: sodium 138 mM, potassium 2 mM, calcium 1.5 mM, bicarbonate 38 mM, acetate 3 mM, magnesium 0.5 mM and glucose 100 mg/dL. Dialysate temperature was 36°C. Anticoagulation was achieved using nadroparin calcium 5700 units (Patient 1) and unfractionated heparin 8000 units (Patient 2).

***Sample handling and analysis***

Samples were collected during a routine 4 hour-HD session. Blood samples for HCQ measurements were drawn from the catheter 2 hours and 1 hour before, and at the initiation of the HD session. During the HD session, pre-dialyzer blood samples were drawn hourly. Post-dialyzer blood samples were drawn at 1 and 3 hours. Spent dialysate HCQ measurements were made in post-dialyser effluent flow samples drawn hourly and in the total ultrafiltration volume collected in a container at the end of HD session, after stirring its content. Additional blood samples for HCQ measurements were drawn at 1 and 2 hours after the end of HD session to measure the rebound. Urinary excretion of HCQ in Patient 2 was calculated on a 24-hours urine sample on the day before the experiment.

Collected samples were directly sent for analysis. Blood HCQ concentrations was measured at the Laboratory of Analytical Chemistry of the Cliniques universitaires Saint-Luc, Department of Clinical Chemistry, by a validated liquid chromatography tandem mass spectrometric (LC-

MS/MS) method, on a Waters Xevo TQ-S micro, in ESI+ mode (Waters, Milford, Ma, USA). Red blood cells were hemolysed by methanol containing HCQ-d4, used as internal standard. The resulting sample underwent a liquid-liquid extraction using dichloromethane/ether/hexane (30/50/20) + 0.5% methylbutan-1-ol, at a basic pH with borate buffer. The next step was the evaporation of the organic phase followed by the reconstitution of the dry residue in the mobile phase. The reconstituted sample was injected on a BEHC18 UPLC column, 2.1 x 100, 1.7µm from Waters. The mobile phase consisted of a buffer ammonium formate 5mM at pH3 with acetonitrile + 0.1% formic acid. Dialysate and urine samples were processed in the same way, calibrations curves were prepared in blank dialysate and blank urine to determine HCQ concentrations in these matrixes.

***Pharmacokinetic analysis***

The following formulas were used:

- The total mass of HCQ removed by HD (XHp) was obtained as the sum of the calculated HCQ mass in the post-dialyzer effluent flow (hourly concentration x dialysate flow x 60 min) and the calculated HCQ mass in the ultrafiltration volume (final concentration x final volume), according to the following equation:

$$XH_p = CU \cdot VU + CD_{t1} \cdot VD_{t0-1} + CD_{t2} \cdot VD_{t1-2} + CD_{t3} \cdot VD_{t3-4} + CD_{t4} \cdot VD_{t3-4}$$ [LL1]

  where CU is the HCQ concentration in the ultrafiltration volume, VU is the total ultrafiltration volume, CD is the HCQ concentration in the efferent dialysate, VD is the volume of spent dialysate and t is the time interval at which measures were made (in hours).

- Dialytic clearance was calculated by dividing the total HCQ mass removal by the area under the drug concentration versus time curve during dialysis.

- Rebound (Rbd) was calculated using the following equation:

  $Rbd\ (\%) = C_{after} - C_{end} / C_{end} \times 100\%$

## Results

Patient 1 was a 35-year-old African woman on in-center maintenance HD for 4 years for end-stage kidney disease (ESKD) secondary to a SLE, with no residual renal function. Her vascular access was a tunneled right internal jugular catheter, and she was dialyzed 4 hours three times weekly. Kt/V was 1.2. She weighed 62 kg for 162 cm. She had been treated with HCQ 200 mg/48h for ten years with a good tolerance. Patient 2 was a 50-year-old Caucasian man on in-center maintenance HD for the last 2 years over a tunneled right internal jugular catheter (Kt/V 1.2). He had a residual diuresis of 650 mL/24h (average of creatinine and urea clearance 2.4 mL/min). He weighed 94 kg for 175 cm and had been on HCQ 200mg/24h for the last six months without any side effect. HCQ blood level was at the therapeutic range (500-1500 ng/mL) in Patients 1 and 2 (1003 and 595 ng/mL, respectively) before starting the present study. [FS2] The last HCQ dose was taken 24 hours before the beginning of the experiment.

Characteristics of the HD session are depicted in Table 1. As depicted in Table 2 and Figure 1, blood HCQ concentration was in the therapeutic range and stable before starting the HD session, reflecting complete distribution of the last dose. Only slight fluctuations of HCQ blood levels occurred during the HD session. HCQ concentration in the dialysate was very low, close to the lower limit of detection (10 ng/mL) (Table 2). The two-hours post-dialysis rebound was small (11 and 9% in Patients 1 and 2, respectively).

During the HD session, 814 and 1487 µg of HCQ were removed in Patients 1 and 2, respectively. Dialytic clearance was calculated at 3.8 mL/min (Patient 1) and 11.6 mL/min (Patient 2). Daily urinary elimination of HCQ in Patient 2 was measured at 492 µg.

## Discussion

This is the first study assessing the efficacy of HD in HCQ clearance. HCQ appears to be almost not dialyzable. Recently, Giaime *et al.* measured HCQ plasma levels before and after HD

session, in 21 maintenance HD patients with SARS-CoV-2 infection treated with high doses (600 mg/day) of HCQ for 3-10 days (6). Plasma HCQ concentrations decreased by 76% in average after 3 hours of HD, suggesting a significant HCQ removal. However HCQ clearance was actually not measured and the time between HCQ intake and dialysis was not mentioned. Moreover, HCQ concentrations were measured on plasma instead of whole blood, which is known to be unreliable (5). On the contrary, our results clearly show that HD is inefficient in clearing HCQ from blood. Of note, it is recommended that HCQ concentration be measured in whole blood rather than in plasma, because HCQ tends to strongly accumulate in blood cells (blood-to-plasma ratio around 7) (5). Given its small molecular weight, its hydrophilic characteristics and its significant, therapeutic blood levels measured before the HD session, a considerable HD removal could be expected. However the quantity of HCQ retrieved in the spent dialysate and the ultrafiltrate was very low in our study. This finding, along with the extralong half-life of HCQ, fits with an extensive tissue uptake rather than with an inability to clear the drug from the plasma.

We aknowledge that the main limitation of our study is the small number of included patients. However, the reproducibility of the measures within the patients and between the two patients is clear. Second, it is unknown whether our results are extrapolable to hemodialfiltration. Yet, the mass of HCQ removed by convective gradient and found in the ultrafiltrate was negligible, which strongly argues against a potential impact of hemodialfiltration on the dialyzability of this drug.

Our study has several strengths. First, HCQ measurements were performed in blood and post-dialyser and ultrafiltration effluent samples at predetermined regular intervals during the HD session, allowing a detailed view of the behaviour of HCQ throughout the whole duration of the procedure. Second, additional blood samples for HCQ measurements were drawn at 1 and 2 hours after the end of HD session to determine the rebound phenomena. Third, the last HCQ

tablet was taken 24 hours before the first blood sample by both patients, with the aim to allow complete drug distribution and thus avoid fluctuations related to the drug absorption (7,8). Indeed, starting HD prior to distribution equilibrium might result in increased removal of drug compared to what would be found in the real clinical world (8). Importantly, HCQ blood-to-plasma ratios tend to be lower at very early times following intravenous HCQ infusion, which suggests that distribution from plasma into blood cells is not rapid (5). Our two patients had been taking HCQ at stable doses for several months-years, and their blood HCQ levels were stable, at therapeutic ranges, before starting dialysis, which strongly suggests that drug distribution was complete.

In conclusion, we demonstrate that HCQ should be considered as non-dialyzable. The efficacy of HD in removing the hydrophilic HCQ is likely to be mitigated by its huge volume of distribution. This finding argues against the use of HD in case of acute HCQ overdose. Conversely, no supplementary dose is indicated after HD sessions in patients treated with HCQ. The timing of HCQ dosing is thus independent of the timing of the HD session due to the large intracellular stocks. Our results highlight the importance of blood levels measurement to guide proper, individualized HCQ dosing in maintenance HD patients.

**Compliance with Ethical Standards**

The study protocol was approved by the Ethics Committee of the Cliniques Universitaires Saint-Luc, Université catholique de Louvain (number 2020/24AVR/241). Both patients cited in this paper gave written informed consent to participate in the study.

**Disclosures:**

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For the remaining authors none conflict of interest were declared.

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## Legend to figure

**Figure 1.** Evolution of blood HCQ levels before, during and after HD session in Patients 1 and 2.

**Table 1: HD session characteristics**

| | Patient 1 | Patient 2 |
|---|---|---|
| **Actual HD time (min)** | 240 | 240 |
| **Blood flow rate (mL/min)** | 250 | 290 |
| **Dialysate flow rate (mL/min)** | 250 | 290 |
| **Ultrafiltration rate (mL/h)** | 750 | 625 |
| **Actual total ultrafiltration volume (mL)** | 3000 | 2500 |
| **Urea reduction ratio (%)** | 73 | 72 |

**Table 2. Hydroxychloroquine concentrations over time (ng/mL).**

## A-Patient 1

| | Predialysis | | Perdialysis | | | | | Postdialysis | |
|---|---|---|---|---|---|---|---|---|---|
| **Time (hours)** | **$H_{-2}$** | **$H_{-1}$** | **$H_0$** | **$H_1$** | **$H_2$** | **$H_3$** | **$H_4$** | **$H_5$** | **$H_6$** |
| **Predialyzer blood** | 886 | 886 | 946 | 882 | 914 | 924 | 771 | 860 | 868 |
| **Postdialyzer blood** | | | | 852 | | 885 | | | |
| **Dialysate** | | | | 16 | 12.2 | 10.4 | 10 | | |
| **Ultrafiltrate** | | | | | | | 11.6 | | |

## B-Patient 2

| | Predialysis | | Perdialysis | | | | | Postdialysis | |
|---|---|---|---|---|---|---|---|---|---|
| **Time (hours)** | **$H_{-2}$** | **$H_{-1}$** | **$H_0$** | **$H_1$** | **$H_2$** | **$H_3$** | **$H_4$** | **$H_5$** | **$H_6$** |
| **Predialyzer blood** | 533 | 489 | 554 | 556 | 527 | 587 | 550 | 651 | 599 |
| **Postdialyzer blood** | | | | 515 | | 497 | | | |
| **Dialysate** | | | | 20.3 | 20.2 | 20.9 | 15.3 | | |
| **Ultrafiltrate** | | | | | | | 21.3 | | |

**Ref. H: hour.**