Supplemental Digital Content

Appendix A

Study hypotheses

Generally, water transport across a capillary can be expressed with the following equation:

$$\frac{dVp}{dt} = RF(t) - UF \tag{A.1}$$

where UF is the ultrafiltration rate (in our case constant), and RF is the plasma refilling flow rate. RF may be expressed using Equation A.1 as:

$$RF = \frac{dVp}{dt} + UF \tag{A.2}$$

UF is measured online my the dialysis machine and the rate of change of plasma volume, dVp/dt, is calculated as the rate of change of blood volume dVB/dt, assuming that the volume of erythrocytes is constant during the dialysis session. The proof comes from the fact that blood volume VB is the sum of plasma volume Vp and the volume of cellular elements of blood Vc:

$$VB = Vp + Vc \tag{A.3}$$

Because Vc is constant in absence of osmotic fluid shifts, therefore $\frac{dVc}{dt} = 0$ and $\frac{dVB}{dt} = \frac{dVp}{dt}$. Using $\frac{Ht_0}{Ht(t)} = \frac{VB(t)}{VB_0}$ one derives a formula for dVp/dt:

$$\frac{dVp}{dt} = VB_0\left[\frac{d}{dt}\left(\frac{Ht_0}{Ht(t)}\right)\right] \tag{A.4}$$

where Ht(t) and Ht_0 are hematocrit values at time t and time t = 0. Thus, one can calculate the absolute plasma volume change rate, dVp/dt, from pre-HD blood volume, VB_0, and relative hematocrit change.

The refilling flow rate RF is the sum of transcapillary fluid flow described by the Starling equation and lymphatic flow, L:

$$RF = L_p[(\Pi_c - \Pi_i) - (P_c - P_i)] + L \tag{A.5}$$

where Π_c, Π_i, P_c, P_i are the capillary and interstitial oncotic pressure and capillary and interstitial hydrostatic pressure, respectively, and L_p is the transcapillary filtration coefficient [1, 2]. In equilibrium conditions (prior to the start of HD), RF = 0 and:

$$(\Pi_c(0) - \Pi_i) - (P_c - P_i) + L / L_p = 0 \tag{A.6}$$

and therefore $\Pi_c(0)=\Pi_i+P_c-P_i-L/L_p$. Assuming that pressures are constant, and that lymph flow is much smaller than vascular refilling rate, the equation (A.5) can be simplified to::

$$RF = L_p\left(\Pi_c(t) - \Pi_c(0)\right) \tag{A.7}$$

The assumption that only plasma oncotic pressure changes during HD session is a simplification, as the changes in all other variables may be expected. However, if the overall, concerted impact of their changes on the refilling rate is smaller than the effect in the change of plasma oncotic pressure, then Equation A.7 may be considered approximately correct. However, because of the uncertainty of the degree of approximation, it is better to keep the name “refilling coefficient”, for the variable calculated by this method based on the combination of Equations A.2 and A.7:

$$Kr(t) = \frac{\frac{dVp}{dt} + UF}{\left[\Pi(t) - \Pi(0)\right]} \tag{A.8}$$

Thus, Kr represents the refilling flow rate as hypothetically driven by the increase in plasma oncotic pressure, and can be considered a measure of efficiency of the refilling mechanism.

Appendix B

Data processing

An exponential function of the form $\mathrm{VB_r(t) = Ae^{-Bt} + C}$, was fitted to the measured relative blood volume change (VB_r), with A, B and C being parameters estimated through least-squares method, where C is the steady state blood volume when refilling balances ultrafiltration, A+C is the initial blood volume, and B is the rate constant for the decrease of blood volume (the average relative error of the fitting, calculated as the square root of the average of all the quadratic errors between experimental and simulated data divided by the number of experimental points, was 0.005 ± 0.002). Modelling VB_r with a smooth algebraic function allowed for easy calculation of its derivative, which is requested by the model [2]:

$$\frac{\mathrm{dVB_r}}{\mathrm{dt}} = -\mathrm{ABe^{-Bt}} \qquad \text{(B.1)}$$

The estimation of the initial blood volume (L) was based on the following anthropometric formula [3]:

$V_0 = 0.0285H + 0.0316BW - 2.82$ (for males), $V_0 = 0.01652H + 0.03846BW - 1.369$ (for females), where *BW* (kg) is the body mass and *H* (cm) is the height of the patient.

Since such formula is apt to estimate the blood volume of a euvolemic patient, we chose to calculate first the post-HD blood volume (assumed to be related to the “dry volume” of the patient) and then used the information about the percentage blood volume decrease during the session to estimate the initial blood volume. However, the actual blood volume at the end of dialysis session may be lower than the “equilibrium” blood volume at dry body weight because refilling continues after dialysis before a stable blood volume is reached [1, 4]; therefore our approach may also yield an overestimated blood

volume, but the error is probably smaller than if the “dry weight” formula is applied for the estimation of blood volume of fluid overloaded patients.

Oncotic pressure Π was calculated using the Landis-Pappenheimer formula [5]:

$$\Pi = 2.1\mathrm{Cp} + 0.16\mathrm{Cp}^2 + 0.009\mathrm{Cp}^3 \qquad \text{(B.2)}$$

where Cp is plasma total protein concentration (g/dL).

Another exponential function $\Pi(t) = F - De^{-Et}$ was used to interpolate the oncotic pressure data. D, E and F are the least-squares parameters of the curve. The average relative error of the fitting, calculated as the square root of the average of all the quadratic errors between experimental and simulated data divided by the number of experimental points, was 0.46 ± 0.27 mmHg). The application of such function allows to describe with the same formula approximately linear profiles as well as those with a clear trend to equilibration [6].

Kr was then calculated from Equation A.8 as:

$$\mathrm{Kr}(t) = \frac{-ABe^{-Bt} + UF}{D(1 - e^{-Et})} \qquad \text{(B.3)}$$

Appendix C

F-cell ratio bias

The F-cell ratio is defined as $F(t) = Ht_{WB}(t)/Ht_{MC}(t)$, where Ht_{MC} is the hematocrit in the systemic (macro) circulation and Ht_{WB} is the whole-body hematocrit, i.e. an average of hematocrit values in micro and macro circulations [7]. The methods of assessing blood volume that rely on hematocrit measurements from the macrocirculation (like online optical absorption) could underestimate the final blood volume loss by almost 50% in comparison to the golden standard (such as the marker dilution method). In this study the data [7] were used to calculate the F-cell ratio correction for the relative blood volume data, in order to get an estimation of how much Kr would be influenced by this effect. A linear function was obtained from the published values of F(t) that corresponded to the patient with the highest difference between whole-body and macrocirculation hematocrit [7]. From the definition of F(t):

$$Ht_{MC}(t) \cdot F(t) = Ht_{WB}(t) \tag{C.1}$$

and therefore

$$\frac{Ht_{MC}(t)}{Ht_{MC}(0)} \cdot \frac{F(t)}{F(0)} = \frac{Ht_{WB}(t)}{Ht_{WB}(0)} \tag{C.2}$$

Because at the start of dialysis $Ht_{WB}(0) = F(0) \cdot Ht_{MC}(0)$.

Using the corrected value of the relative hematocrit drop $\frac{Ht_{WB}(t)}{Ht_{WB}(0)}$, one can proceed as described in Appendix A (formula A.4) to calculate the rate of blood volume change and the refilling rate.

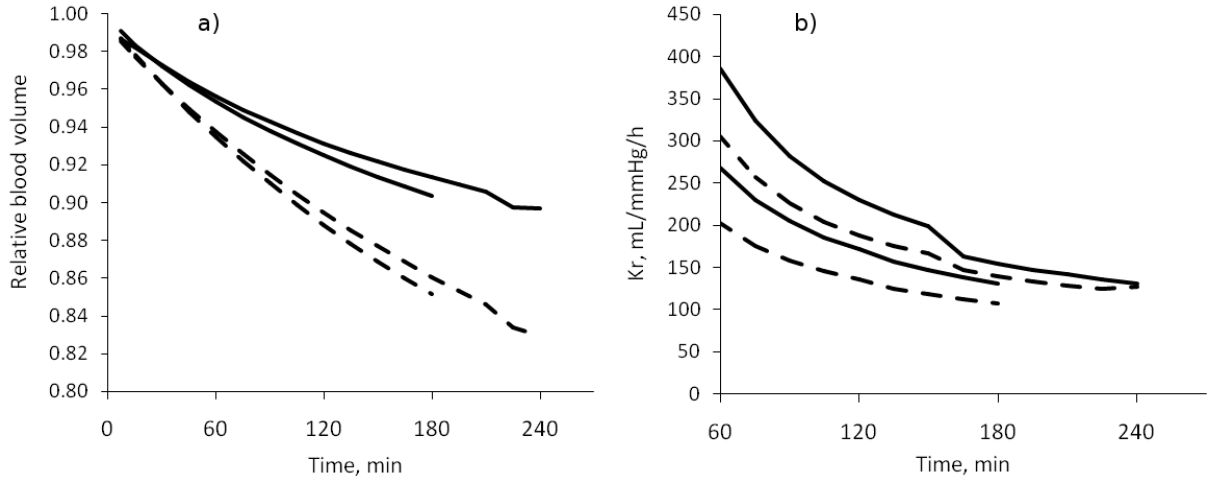

Figure C1. a) Relative blood volume changes in short and long hemodialysis sessions, before (continuous lines) and after F-cell ratio correction (dashed lines). **b)** Refilling coefficient in both sessions calculated from uncorrected (continuous lines) and corrected blood volume values (dashed lines).

Bibliography

1. Schneditz D, Roob J, Oswald M et al. Nature and rate of vascular refilling during hemodialysis and ultrafiltration. *Kidney Int* 1992; **42**: 1425-33.
2. Tabei K, Nagashima H, Imura O et al. An index of plasma refilling in hemodialysis patients. *Nephron* 1996; **74**: 266-74.
3. Lentner C, ed. Geigy Scientific Tables: Physical Chemistry, Composition of Blood, Hematology, Somatometric Data. 8th ed. Basle: Ciba-Geigy Limited 1984.
4. Leypoldt JK, Cheung AK, Steuer RR et al. Determination of circulating blood volume by continuously monitoring hematocrit during hemodialysis. *J Am Soc Nephrol* 1995; **6**: 214-9.
5. Landis E, Pappenheimer J. Exchange of substances through the capillary walls. *Handbook of Physiology Circulation*. Washington: American Physiological Society 1963:961-1034.
6. Arrigo G, Bucci R, Colasanti G et al. Blood volume modeling and refilling rate estimation in hemodialysis by continuous hemoglobin monitoring. *Int J Artif Organs* 1995; **18**: 509-12.
7. Dasselaar JJ, Lub-de Hooge MN, Pruim J et al. Relative blood volume changes underestimate total blood volume changes during hemodialysis. *Clin J Am Soc Nephrol* 2007; **2**: 669-74.