Summary

Every year more than two million people around the world are treated for end-stage renal disease (ESRD). Chronic kidney disease are closely related to cardiovascular disease, often one leading to the other, and most people with ESRD die as a result of cardiovascular complications. When a kidney transplant is not possible, renal replacement therapies are the only alternative for ESRD patients. All types of dialysis therapy have the aim removing wastes from the blood, preserving its physiological osmotic balance, and regulating the amount of fluid in the body.

Thanks to continuous technological advancements, dialysis patients are today living longer than ever. The longer time spent by patients under dialysis dictated an increasing importance in ensuring a high quality of life, maximizing comfort and minimizing complications. Haemodialysis (HD) is especially stressful for patients, requiring many treatment hours, several times per week. Moreover, HD sessions are prone to complications, such as muscle cramps and hypotensive collapse, requiring constant monitoring of the patients. To minimize occurrence of such complications, it is important to correctly measure and prescribe the adequate dose of dialysis (regime or intensity of the therapy) for every patient, which depends on the frequency of the sessions, their length, intensity of water removal, etc. The outcome of a HD session is dictated also by the reaction of the body, specific for each patient. This response depends on complex mechanisms such as plasma refilling, lymphatic absorption, electrolytes kinetics, transport of proteins. To quantify the efficacy of these mechanisms is not straightforward: many of the variables involved are difficult to measure without discomfort for the patient or are not possible to measure directly at all.

Mathematical models can be useful both to improve our knowledge of the physiological processes taking place during HD and for clinical applications. Tuning the parameters of the model on clinical data is a way to estimate quantities that we cannot measure, and the simulated results can be used to decide the correct dose of dialysis for a patient. The balance between physiological rigor and clinical applicability is important in the development of mathematical models: the more rigorous a model is, the less abstract its parameters will be, at the cost of increased complexity.

Objective of this dissertation was the development of a model of fluid and solutes transport during HD as physiologically accurate as possible, but still simple enough to be used as a clinical tool. An important step was the description of physiological mechanisms of importance for assessment of fluid distribution during dialysis, like the relationships between interstitial volume and pressure, and the contribution of the lymphatic absorption to plasma refilling, which are usually overlooked in models of HD. Greater detail was also reserved for the description of two main interfaces in the mode: the capillary wall and the cellular membrane, using porous membrane transport theory for the former, and an explicit representation of the sodium-potassium pump for the latter.

The ‘blocks’ with which the model was built were chosen discussing different existing approaches, in order to select the best compromise between simplicity and physiological soundness. All the model implementations and analysis described were applied to data collected in standard HD sessions, comprehensive of several types of measurements.

One of the main complications of HD is intradialytic hypotension: the sudden collapse in blood pressure that happens in over 20 % of all dialysis sessions, and severely hinders both the short and long term efficacy of the treatment. It has been shown that intradialytic hypotension is strictly correlated with high

ultrafiltration rates and insufficient plasma refilling in the patient; modelling the refilling mechanism was thus a fundamental step. A two-compartment model was proposed, describing the transport of water and proteins between interstitium and plasma, across the capillary membrane. The transport pathways through the membrane were described using the 3-pore model theory, which is of common use in the field of kinetic modelling of peritoneal dialysis, but not so for HD. This approach assumes that the transport of all substances takes place through pores of different sizes (large, small, and ultrasmall), theoretically corresponding to different physiological pathways present in the endothelium. The flows of solutes and fluid, for each pathway, were calculated according to Kedem and Katchalsky's thermodynamic description of transport across semipermeable membranes and the Starling equation applied to HD. Clinical measurements of relative blood volume changes, haematocrit, fluid compartments volume and plasma total protein concentration, were used as starting points for the simulations. The model was fitted to the clinical data to estimate two unknown parameters, the hydraulic permeability of the membrane, *Lp*, and the fraction of such permeability attributed to the large pores, α_{LP}; all the other parameters of the model were either measured empirically in previous physiological studies, or calculated internally imposing assumptions on the steady-state conditions of the system. The model introduced new features compared to similar, previously published models of refilling in HD, such as detailed volume–hydraulic pressure–lymphatic flow relationships for interstitial fluid.

Upon identification of the optimal parameters (in good accord with literature) the model was able to reproduce the clinical profiles of plasma volume and total protein concentration with good accuracy, less than 2 % root mean squared error. The impact of using different values for several parameters which cannot be easily measured, and were taken from the literature, was discussed in the detail. For some of them (fractional contribution of small pores to *Lp*, radius of small pores, lymph flow sensitivity to changes in interstitial pressure) different values resulted in the expected changes to closely-related quantities; changing the ratio of interstitial-to-plasma protein concentration resulted in many important changes in various parameters and variables, including the calculated values of capillary hydraulic pressure and lymph flow. Despite the estimated optimal parameters and the variables compared to the data changed little, these results suggested that the choice of such parameters can strongly alter the predicted internal physiological processes.

The filtration coefficient of the capillary wall, *Lp*, the main determinant of the intensity of the fluid flows described in the model, was further discussed. Due to the impossibility of measuring an average whole-body value for a patient, when modelling refilling *Lp* is usually estimated from the data or assumed a priori; however, a method for easily calculating *Lp* from clinical data of relative blood volume, haematocrit, and protein concentration was proposed in the past. It was suggested that, under particular assumptions, *Lp* could be calculated as the ratio between plasma refilling rate and increase in oncotic pressure from the start of HD and this estimate was called *refilling coefficient*, *Kr*; it was observed that the value of *Kr* calculated in this way was decreasing throughout the session. The assumptions necessary to calculate *Kr* are that the generation of a refilling flow during HD is caused by the increase in plasma oncotic pressure, while changes in the remaining Starling forces and lymphatic flow are essentially negligible.

This work reports the results of applying the method for calculating *Kr* to data coming from two HD sessions with different pre-dialytic interval, initial fluid overload, and duration of treatment, in order to compare the behaviour of this parameter in different conditions. The refilling rate during HD was estimated from the difference between the ultrafiltration rate and the rate of change of plasma volume, calculated from online measurements of relative blood volume changes; serum total protein concentration

was collected from hourly blood samples. The decreasing profile of *Kr*, already reported in literature, was confirmed in the new patients examined; on average its value decreased from 5.5 mL/min/mmHg at 1 hour to 2.4 mL/min/mmHg at the end of the treatment. The kinetics of *Kr* was similar in the two sessions: the final value was reached in both cases around 3 hours, and after it was essentially stable. The final values were similar in both sessions. Despite higher refilling rates at 1 h in the session with higher pre-dialytic fluid overload, *Kr* at 1 h was not significantly different; however, a subgroup of two patients was individuated with remarkably high difference in initial *Kr* between the two sessions. These results suggest that *Kr* is in general relatively insensitive to differences in initial fluid status in an HD patient, and exceptions to this can be helpful in highlighting subjects with particularly high sensitivity to initial fluid status changes; however, further investigation should be carried out in this direction.

The two-compartment model of plasma refilling was used to test *Kr* assumptions of the predominance of changes in capillary oncotic pressure over the remaining Starling forces. The model allowed calculating changes in the interstitial pressures (oncotic and hydraulic) and lymphatic flow. The subsequent correction in the equation of the refilling coefficient ultimately showed that the decreasing behaviour of the parameter *Kr* was caused by the assumptions ignoring the non-negligible effect of the other Starling forces. However, a subgroup of patients was observed that was characterized by values of initial *Kr*, for which the effect of the interstitial pressures and lymphatic flow was not enough to justify entirely the decrease in *Kr* during dialysis: it is possible that the behaviour of this parameter is caused by the combination of error introduced by faulty assumptions and, in some cases, additional physiological mechanisms (e.g. changes in vasodilation).

The balance of electrolytes across the cellular membrane is as important, for the study of fluid distribution during HD, as the modelling of refilling mechanisms. The kinetics of the most abundant ionic solutes in the extra- and intracellular fluids, sodium and potassium, respectively, was studied using different single species modelling, to decide which approach could be better implemented in a larger model with refilling and lymphatic mechanisms. Two relatively simple models were considered: pseudo-one compartment and two-compartment, both fitted to clinical data. In the former, the solute is distributed in one accessible compartment, which is initially in equilibrium with an indefinite, inaccessible compartment that acts as reservoir of the substance. This approach functions like a ‘black box’, focusing only on reproducing accurately clinical data, but has been shown to be effective. Two parameters are estimated in this case, the initial volume of the accessible compartment, and the diffusive mass transfer coefficient which regulates the transport between the two compartments. The two-compartment model describes transport between extracellular and intracellular compartments, both with passive diffusion and, in the case of sodium and potassium, with active transport by an ATPase pump. Only one parameter was estimated, the maximum transport rate of the pump, Jp_{MAX}. The models were not able to accurately identify the transport parameters for sodium, probably because of the often small variation in sodium concentration in the clinical profiles. Good results were instead obtained with potassium, and the optimal parameters were for both models in good agreement with previous studies. Even though it was shown that the pseudo-one compartment model could slightly better reproduce the data, the two-compartment model was deemed a better candidate for implementation in a bigger model because of the more physiologically accurate assumptions.

The final, comprehensive model described the transport of fluid, proteins, sodium, potassium and urea. The model has three compartments for water, and two compartments for each solute. Proteins move between interstitial and vascular compartment, and do not cross the cell membrane. Smaller solutes have similar concentration in interstitial fluid and plasma, and these are thus grouped into the extracellular

compartment for the description of small solutes transport. Transport through the capillary membrane was assumed to follow the 3-pore model; sodium and potassium were exchanged both via passive leaks and through the ATPase pump mechanism; urea was added to the model because it represents an important component of total osmolarity in HD patients. The water transport across the cell membrane was proportional to the gradient of total osmolarity between the two compartments. The three unknown parameters estimated were α_{LP}, Lp, and Jp_{MAX}; their values were in line with findings in similar studies. The model was able to describe the clinical data with good accuracy in most of the patients, with small relative errors for almost all of the measured clinical variables. The estimation of Jp_{MAX} was characterized by higher scattering of the values; the scarcity of mathematical models proposed for HD which explicitly represent active transport of sodium and potassium made it difficult to draw comparisons.

Few different versions of the model were implemented, to discuss alternative approaches in modelling its key aspects. The effect of using simplifications in the description of intracellular volume changes was tested, assuming in one case that the volume changed linearly during dialysis (according to volumetric data measured with bioimpedance) and, in the second case, that volume remained constant. Both alternative versions did not significantly improve or worsen the accuracy of the model predictions; the drawback of these simplified descriptions was that the estimation of Jp_{MAX} was affected, with more variability in the results and difficulty to find an optimal value in a higher number of cases.

The choice of the small solutes modelled was dictated by the availability of the clinical data used to assess the accuracy of the simulations; nevertheless, proper implementation of the osmotically driven water transport across the cell membrane might require a higher number of solutes to be described. One additional species, representing anions, was added in an expanded version of the model, to evaluate eventual improvements in the model results. Whereas slightly better accuracy was observed in the simulated profiles of plasma sodium concentration and intracellular volume, the biggest change was once again in the parameter Jp_{MAX}. This parameter was thus confirmed to be very sensitive to changes in the assumptions made on the transport through the cell membrane. This observation means that the physiological interpretation of Jp_{MAX} values identified in such models is to be considered in close relation to the complexity of the model. It was also observed that including additional ionic solutes might require the implementation of the description of electrical effects in order to model the equilibrium of such substances.

In conclusion, the baseline version of the final model proposed in this dissertation had the merit of being able to reproduce accurately individual patients' data with the estimation of few parameters, striking a good balance between representing important physiological mechanisms and avoiding the introduction too much complexity in the model.