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2. Tamme K, Oselin K, Kipper K, Tasa T, Metsvaht T, Karjagin J, Herodes K, Kern H, Starkopf J. Pharmacokinetics and pharmacodynamics of piperacillin/tazobactam during high volume haemodiafiltration in patients with septic shock. Acta Anaesthesiol Scand. 2015; doi: 10.1111/aas.12629

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ABBREVIATIONS

%SE	percent of standard error
AKI	acute kidney injury
ALT	alanine aminotransferase
APACHE II	acute physiology and chronic health evaluation II score
AST	aspartate aminotransferase
AUCτ	area under the concentration-time curve during the dosing interval
BLBLI	β-lactam-β-lactamase inhibitor combination
CHF	continuous haemofiltration
CI	95% confidence interval
CL	total body clearance
CLcr	creatinine clearance
Cmax	maximal concentration
CRP	C-reactive protein
CRRT	continuous renal repalecement therapy
CVVHDF	continuous veno-venous haemodiafiltration
CVVHF	continuous veno-venous haemofiltration
EGF	epidermal growth factor
eGFR	estimated glomerular filtration rate
EHVHF	extra high volume haemofiltration
ESRD	end-stage renal disease
EUCAST	European Committee of Antimicrobial Susceptibility Testing
Hb	haemoglobin
Hct	haematocrit
HD	haemodialysis
HDF	haemodiafiltration
HF	haemofiltration
HVHDF	high volume haemodiafiltration
HVHF	high volume haemofiltration
ICU	intensive care unit
IFN-γ	interferon γ
IIV	inter-individual variability
IL	interleukin
IQR	interquartile range
Kel	elimination rate constant
MCP-1	monocyte chemoattractant protein 1
MIC	minimal inhibitory concentration
NS	not significant
PD	pharmacodynamics
PK	pharmacokinetic
PTA	probability of target attainment
Q	intercompartmental clearance

Qb	blood flow rate
Qd	dialysis fluid flow rate
Qe	effluent flow rate
Qu	ultrafiltration rate
RRT	renal replacement therapy
RSE	residual standard error
SIRS	systemic inflammatory response syndrome
SLED	sustained low efficiency dialysis
SOFA	sequential organ failure score
SVHF	standard volume haemofiltration
T>MIC	time over minimal inhibitory concentration
T1/2	half-life
TDM	therapeutic drug monitoring
TNFα	tumor necrosis factor α
UF	ultrafiltration
V1	volume of distribution of central compartment
V2	volume of distribution of perpheral compartment
V_D	volume of distribution
VEGF	vascular endothelial growth factor
WBC	white blood cells

1. INTRODUCTION

Sepsis – a systemic, deleterious host response to infection – and its most complicated forms, severe sepsis (acute organ dysfunction secondary to documented or suspected infection) and septic shock (severe sepsis plus hypotension not reversed with fluid resuscitation) (Dellinger et al. 2013), remain a major healthcare problem (Angus et al. 2013). The incidence of severe sepsis is rising (Brun Buisson et al. 2004). Although mortality has decreased during the last decade, it still remains high (Brun Buisson et al. 2004, Dombrovskiy et al. 2007). Acute renal failure, often occurring as a complication of severe sepsis, is an independent risk factor of death (Brun Buisson et al. 2004).

The key issues in the treatment of sepsis are adequate antibacterial therapy, rapid source control and handling of the dysbalance between pro- and anti-inflammatory forces. Delay in initiating antibacterial treatment or inadequate choice of antibiotics have been shown to lead to increased mortality in septic patients (Kumar et al. 2006). Control of pro- and anti-inflammatory forces is equally important, but difficult to achieve due to the highly complicated nature of the reaction and huge number of mediators involved. During the last fifteen years nonselective extracorporeal removal of mediators using renal replacement therapies (RRT) has been suggested as beneficial in restoring immunohomeostasis (Ronco et al. 2004). Removal of middle size molecules i. e. cytokines depends on the modality of the RRT, but also on the filter characteristics and the volume of therapy (Ricci et al. 2006a, Cerda et al. 2009). Clinical and experimental data show improved survival of septic patients with renal failure with increasing dose of RRT (Honore et al. 2000, Schiffl et al. 2002, Cornejo et al. 2006, Bouman et al. 2007). Still some controversy exists as a randomised controlled trial failed to confirm this effect (Joannes-Boyau et al. 2013). Preliminary results from Kron and colleagues suggested that high volume haemodiafiltration (HVHDF) with specific Ultracontrol mode technique is associated with superior survival compared to survival predicted by disease severity scores (Kron et al. 2011). Unlike the standard pressure and volume control, the Ultracontrol mode of filtration titrates the transmembrane pressure during the diafiltration so, that optimal conditions (highest filtrate volume at the lowest possible transmembrane pressure) are achieved and maintained. Use of ultrapure water supplemented with electrolytes instead of incased replacement solutions makes the technique feasible – the costs are almost equal to the intermittent technique (Kron et al. 2011).

While potentially effective in restoring immunohomeostasis, high filtration volumes may lead to excessive removal of antibiotics and ineffective plasma and tissue concentrations of the vitally important drugs, thus decreasing or eliminating the clinical benefits (Jamal et al. 2014b). On the other hand, uncontrolled increase in dosing may result in toxicity (Roberts DM et al. 2012). The pharmacokinetics (PK) of β-lactam antibiotics has been studied during

different methods of RRT, used in septic patients, and significant, RRT dose dependent elimination has been shown (Jamal et al. 2014b).

The present thesis studies the impact of a specific mode of HVHDF – the predilution Ultracontrol – on the PK of two β-lactam antibiotics, and explores the patients' circulatory parameters and inflammatory mediators in response to this treatment.

2. REVIEW OF LITERATURE

2.1 High volume haemo(dia)filtration

In current practise different methods of RRT are used in critically ill patients (Mehta et al. 1999, Ronco et al. 2001, Ricci et al. 2006b, Overberger et al. 2007, Gatward et al. 2008, Legrand et al. 2013, Jamal et al. 2014a, Iwagami et al. 2015). Solute clearance can be achieved either by diffusion (haemodialysis, HD), convection (haemofiltration, HF) or the combination of both (haemodia-filtration, HDF) (Fig. 1) (Pannu et al. 2008). The volume of replacement fluid used determines whether the method in use can be defined as high volume HF or HDF. While small molecules diffuse rapidly and are efficiently removed by haemodialysis, the clearance of middle-sized molecules, such as beta-2-microglobulin and cytokines is believed to be better via haemofiltration (DeVriese et al 1999, Cerda et al. 2009). All these methods can be performed continuously, intermittently or in hybrid forms like sustained low efficiency dialysis (SLED) or extended daily dialysis, where the intermittent procedure is extended to more than 10 hours per day (Palevsky et al. 2013). Continuous renal replacement therapy is the most common form used in critically ill patients, although hybrid forms are becoming more widely used (Vesconi et al. 2009, Jamal et al. 2014a).

2.1.1 Definition of high volume haemofiltration

High volume haemofiltration (HVHF) is not well defined in medical literature. Different definitions exist, resulting in the situation, that the high volume group in some studies may be the low volume group in others (Rimmele et al 2012). The conventional filtration volumes, used in critically ill septic patients are 24–35 mL/kg/h (Borthwick et al. 2013), thus, HVHF was initially defined as continuous haemofiltration with ultrafiltration volumes greater than 35 mL/kg/h (Kellum et al. 2002). This definition has been used as late as 2013 (Borthwick 2013). At present, the most frequently used definition is the Pardubice definition, defining HVHF as continuous high-volume treatment of more than 50 ml/kg/h for 24 h per day and intermittent high-volume haemofiltration with brief, very high-volume treatment at 100–120 ml/kg/h for a short period of 4–8 h, followed by conventional continuous veno-venous haemofiltration (CVVHF) (Honore et al. 2007). Not all definitions of intermittent HVHF incorporate the following conventional RRT method. The second edition of Critical Care Nephrology defines high volume hemofiltration as continuous high volume treatment, providing 50–70 mL/kg/h 24 hours a day, and intermittent hemo-filtration with brief, very-high-volume regimens of 100–120 mL/kg/h for 4–8 hours, sometimes called pulse HVHF (Joannes-Boyau et al. 2009).

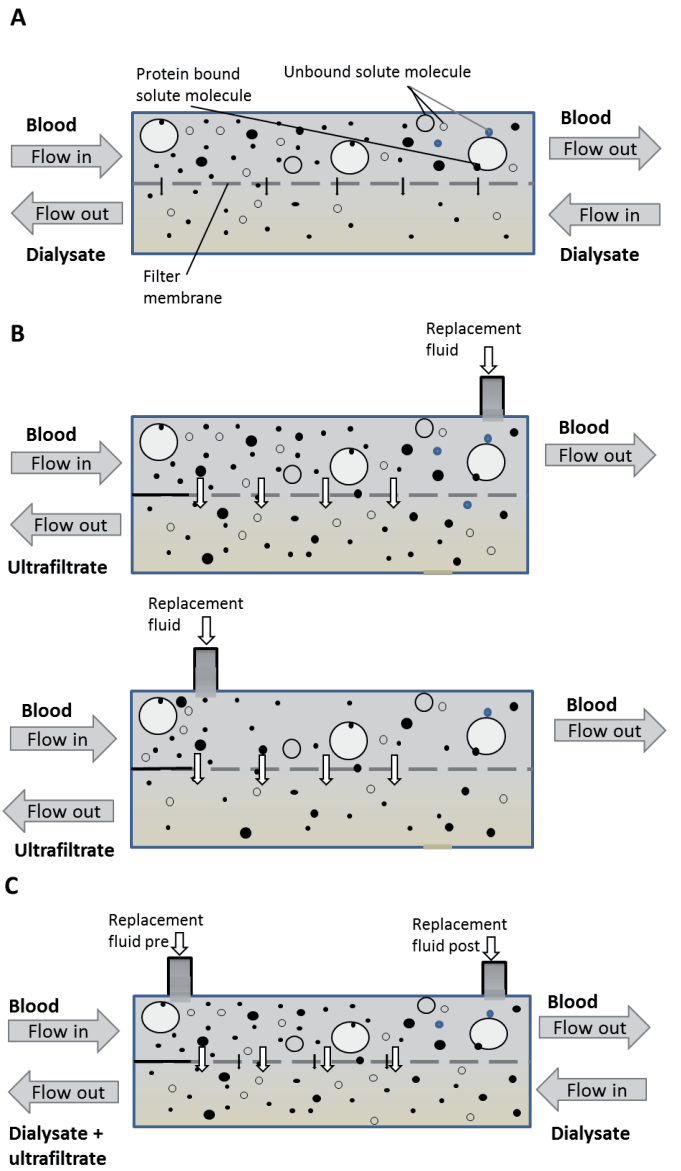

Figure 1. **Principles of solute clearance in RRT. A. Haemodialysis**. The mechanism of solute clearance is diffusion of solutes due to concentration gradient, created by countercurrent flow of blood and dialysate fluid. **B. Haemofiltration.** Solute clearance is achieved by convection due to hydrostatic pressure gradient, generated by dialysis machine across the filter membrane. Both water and solutes are filtered. To avoid excessive fluid loss via filtration, replacement fluid is administered. In postdilution mode (B, upper panel) replacement fluid is administered after the passage of blood over the filter. In predilution mode (B, lower panel) replacement fluid is administered before the passage of blood through the filter and dilutes the concentration of solutes on the filter. The efficiency of solute removal is reduced. **C. Haemodiafiltration.** Combines haemodialysis with haemofiltration, either with pre- or postdilution mode.

2.1.2 High volume haemofiltration for extracorporeal blood purification in severe sepsis and septic shock

Persistently elevated levels of inflammatory markers have been shown to be associated with RRT dependence and death in patients with severe sepsis and septic shock (Payen et al. 2012, Murugan et al. 2014, Murugan et al. 2015). Since the landmark study by Ronco et al. (Ronco et al. 2000), where better survival of critically ill patients with acute kidney injury (AKI) was found with filtration volumes of 35 mL/kg/ h or 45 mL/kg/h, compared to 20 mL/kg/h, higher filtration volumes have been used in septic patients in the hope of removing excess pro- and anti-inflammatory mediators and restoring immuno-homeostasis. Despite that two large multicentre randomised trials, comparing filtration volumes of 20 mL/kg/h to 35 mL/kg/h (VA/NIH Acute Renal Failure Trial Network 2008) and 25 mL/kg/h to 40 mL/kg/h (RENAL Replacement Therapy Study Investigators 2009) did not observe survival benefit, the concept of extracorporeal blood purification in the treatment of sepsis has remained attractive (Ronco et al. 2015).

Animal studies have shown reversal of sepsis or severe acute pancreatitis induced hypotension and decrease in cardiac output by haemofiltration with doses exceeding 50 mL/kg/h (Grootendorst et al. 1992, Grootendorst et al. 1994, Rogiers et al. 1999, Bellomo et al. 2000, Yekebas et al. 2001, Li et al. 2013) with more pronounced effect with increasing filtration volumes (Rogiers et al. 1999).

Clinical studies with high volume haemofiltration are summarised in Table 1. Prospective clinical series with filtration volumes exceeding 50 mL/kg/h in patients with septic shock, refractory to conventional treatment suggested decrease of noradrenaline requirements, stabilisation of haemodynamics and improved survival compared to that predicted by disease severity scores (Oudemans-van-Straaten 1999, Honore et al. 2000, Joannes-Boyau et al. 2004, Cornejo et al. 2006, Ratanarat et al. 2006, Kron et al. 2011, Tapia et al. 2012). High volume haemofiltration was proposed as salvage therapy in intractable septic shock (Honore et al. 2000, Cornejo et al. 2006).

Subsequent randomised clinical trials (RCT-s) reached controversial results. Cole and colleagues compared 8 hour high volume HVHF to 8 hour standard CVVHF and found greater reduction in noradrenaline requirements in the HVHF group (Cole et al. 2001). Some following single centre randomised trials, comparing either pulse or continuous HVHF to standard dose RRT in patients with septic shock or acute pancreatitis showed improved haemodynamics but no survival benefit (Boussekey et al. 2008, Peng et al. 2010, Chu et al. 2013). Other randomised trials have shown no haemodynamic or survival benefit in patients with severe sepsis or septic shock (Bouman et al. 2002, Ghani et al. 2006, Sanchez et al. 2010, Zhang et al. 2012). Škofic and colleagues compared intermittent high volume online haemofiltration to intermittent haemodialysis in critically ill patients with AKI and found no dif-

ferences in mortality between the two groups (Škofic et al. 2012). The biggest multicentre randomised controlled study, comparing CVVHF in doses of 35 mL/kg/h to 70 mL/kg/h was conducted in 18 ICU-s in France, Belgium and the Netherlands. No difference was demonstrated in the primary endpoint of 28-day mortality between the groups. Neither were there any differences in haemodynamic profile, organ failure scores, recovery of renal function, ICU or hospital stay, 60- and 90-day mortality or adverse events, attributable to RRT (Joannes-Boyau et al. 2013). Yet the study was underpowered, recruiting only 30% of the required sample size (Joannes-Boyau et al. 2013).

A Cochrane database systematic review included three randomised controlled studies and concluded that there was very weak evidence to support the use of HVHF in critically ill patients with severe sepsis/septic shock to improve outcomes and that there was no evidence to suggest the treatment intervention was harmful (Borthwick et al. 2013). Another meta-analysis of four randomised trials reached similar conclusion, namely that there was insufficient evidence of a therapeutic benefit for routine use of HVHF for septic AKI, other than on an experimental basis (Clark et al. 2014).

In summary, different methods and doses of HVHF have been studied in different patient groups with controversial results. Although RCT-s have not shown clinical benefit from HVHF, individual patients might still benefit from higher doses of RRT, when hypercatabolic states or sepsis are present (Ronco et al. 2015). A personalised approach, mapping RRT intensity to biomarker levels could be effective (Ronco et al. 2015). The evidence, if and which patient groups would benefit from HVHF remains inconclusive.

Table 1. Clinical studies evaluating the effect of HVHF in treatment of SIRS and shock.

Author year	Study type	Patients, No.	Method of RRT	Main results
Prospective cohort series				
Oudemans van Straaten 1999	Prospective cohort analysis	All ICU patients, treated with HV-HF; sepsis 31%; shock without proven sepsis 67%, 306	Intermittent HVHF UF 5 L/h, postdilution Aim 100 L per procedure	Mortality lower than predicted by disease severity scores
Honore 2000	Prospective interventional	Severe septic shock not responding to conventional therapy, 20	Intermittent HVHF UF 35L/4h	Improved haemodynamic and metabolic response. Mortality lower than predicted. Survivors received higher UF dose per kg body weight
Joannes-Boyau 2006	Prospective inter-ventional	Septic shock 24	Continuous HVHF UF 40–60 mL/kg/h	Haemodynamic improvement. Decrease in noradrenaline requirement Mortality lower than predicted
Cornejo 2006	Prospective inter-ventional	Severe septic shock not responding to conventional therapy, 20	Intermittent HVHF Q_u 100 mL/kg/h	Haemodynamic improvement. Mortality lower than predicted
Ratanarat 2006	Prospective inter-ventional	Severe sepsis 15	Pulse daily HVHF Q_u 85 mL/kg/h	Decrease in noradrenaline requirement
Kron 2011	Prospective inter-ventional	Consecutive ICU patients with mortality risk >50% 21	Extended daily HVHDF Mean Q_u 208 mL/kg/h	Mortality lower than predicted
Tapia 2012	Prospective cohort	Severe septic shock not responding to conventional therapy 31	Intermittent HVHF 6 h Mean Q_u 57 mL/kg/h	Decrease in noradrenaline requirement Mortality lower than predicted

Table 1. Continuation

Author year	Study type	Patients, No.	Method of RRT	Main results
Prospective randomised studies				
Sanchez-Izquierdo 1997	Rando-mised	Critically ill trauma 30	Continuous HVHF (Q_u 7.8 L/h) vs no RRT	Substantial amounts of TNF-α and IL-6 in ultrafiltrate, no difference in serum concentrations compared to controls
Cole 2001	Rando-mised cross-over	Septic shock 11	HVHF (Q_u 6 L/h) 8 h vs conventional HF (Q_u 1 L/h) 8 h	Greater reduction in noradrenaline requirement in HVHF group Greater reduction in complement C3a and C5a serum levels
Bouman 2002	Rando-mised, 2-centre	Intensive care patients with oliguria 106	Early HVHF (median Q_u 48 mL/kg/h) vs early LVHF (median Q_u 20 mL/kg/h) vs late LVHF (median Q_u 19 mL/kg/h)	No benefit in terms of 28-day mortality or improved renal recovery
Ghani 2006	Rando-mised	Severe sepsis and septic shock 33	HVHF (6 h, Q_u 100 mL/kg/h) vs conventional HF (Q_u 35 mL/kg/h)	Decrease in serum IL-6 in HVHF group
Saudan 2006	Rando-mised	Critically ill with AKI 206	CVVHDF Q_e 2.5–4 L/h vs CVVHF Q_u 1–2.5 L/h	Increased 28-day and 90-day survival in CVVHDF group
Boussekey 2008	Rando-mised	Septic shock 20	CHVHF (Q_u 65 mL/kg/h) vs standard HF (Q_u 35 mL/kg/h)	Greater reduction in noradrenaline requirement in HVHF group
Peng 2010	Rando-mised	Severe sepsis 22	Pulse HVHF (Q_u 85 mL/kg/h) for 72 h vs conventional HF (Q_u 35 mL/kg/h)	Greater reduction in noradrenaline requirement in HVHF group, Greater decrease in plasma cytokines concentration

Table 1. Continuation

Author year	Study type	Patients, No.	Method of RRT	Main results
Sanchez 2010	Rando-mised	Septic shock 30	CHVHF (Q_u > 55 mL/kg/h) vs conventional HF (Q_u 35 mL/kg/h)	No difference in haemodynamic parameters or vasopressor requirements between groups 28-day survival 86.7% (HVHF) vs 53.3% (control) (p=0.46)
Zhang. 2012	Rando-mised	Severe sepsis 208	Continuous EHVHF (Q_u 85 mL/kg/h) vs HVHF (Q_u 50 mL/kg/h)	No survival benefit at 28 or 90 days. No benefit in terms of haemodynamic stabilisation
Škofic 2012	Rando-mised	ICU patients with AKI (80% septic) 273	Intermittent HVHF (median Q_u 4.8 L/h) vs intermittent HD (Q_d 500 mL/min)	No difference in mortality or renal recovery
Chu 2013	Rando-mised	Severe acute pancreatitis 30	Pulse HVHF (6 h, Q_u 85 mL/kg/h), followed by con-ventional CHF vs conventional CHF (Q_u 35 mL/kg/h)	Greater reduction in dopamine requirement in HVHF group Greater decrease in plasma cytokines concentration
Joannes-Boyau 2013	Rando-mised, multicentre	Septic shock 140	CHVHF (Q_u 70 mL/kg/h) vs continuous SVHF (Q_u 35 mL/kg/h)	No difference between groups in terms of 28-day mortality, haemodynamic stabilisation or organ function

CHF – continuous haemofiltration, CHVHF – continuous high volume haemofiltration, CVVHDF – continuous high volume haemodiafiltration, EHVHF – extra high-volume haemofiltration, HD – haemodialysis, HF – haemofiltration, HVHF – high volume haemofiltration, IL-6 – interleukin-6, LVHF – low volume haemofiltration, Q_d – dialysate flow rate, Q_e – effluent flow rate (ultrafiltrate + dialysate flow rate), Q_u – ultrafiltrate flow rate, SVHF – standard volume haemofiltration

2.1.3 Removal of pro- and anti-inflammatory mediators by high volume haemofiltration

High volume haemofiltration is an attractive therapy to remove a wide range of pro- and anti-inflammatory molecules that play an important role in the pathogenesis of sepsis (Rimmele et al. 2012). These mediators are mostly water soluble, circulating freely in plasma and have molecular mass ranging from 5 kDa to 60 kDa. Molecules below the haemofiltration membrane cut-off will be filtrated through the membrane together with water and other solutes (Rimmele et al. 2012). Convection is more effective in removing middle molecules than diffusion (De Vriese et al. 1999, Kellum et al. 1998, Cerda et al. 2009). In addition, most haemofiltration membranes have some adsorptive capacities and mediators with molecular weight above the membrane cut-off may be adsorbed on the membrane (Rimmele et al. 2012).

Animal studies have shown that mediators are filtrated through the haemofiltration membrane as the ultrafiltrate from septic animals induces haemodynamic changes similar to septic shock and even death when infused to healthy animals (Grootendorst et al. 1993, Lee et al. 1993, Rogiers et al. 1999). It is not very clear, which cytokines are removed most effectively. Yekebas et al. measured substantial amounts of TNF-α and IL-10 in ultrafiltrate in porcine pancreatitis model (Yekebas et al. 2001), while other studies found very low levels or no TNF-α in ultrafiltrate (Rogiers et al. 1999, Bellomo et al. 2000). A recent meta-analysis of animal studies reached the conclusion that HVHF, but not standard RRT has the potential to achieve appreciable IL-6 and IL-10, but not TNF-α clearances (Atan et al. 2013a).

The results of human studies are similarly controversial. Not many studies have measured cytokine concentrations in plasma or ultrafiltrate. Some human studies have shown significant decrease in plasma cytokine concentrations in patients treated with HVHF, namely IL-10 (Cole et al. 2001), IL-6 (Ghani et al. 2006) or TNF-α, IL-1, IL-4, IL-6 and IL-10 (Peng et al. 2010) in patients with severe sepsis, and TNF-α, IL-6 and IL-10 in patients with severe acute pancreatitis (Chu et al. 2013). Sanchez-Izquierdo et al. found substantial amounts of TNF-α and IL-6 in ultrafiltrate in critically ill trauma patients, but no significant decrease in serum cytokine levels and no difference compared to not HVHF treated controls (Sanchez- Izquierdo et al. 1997). A recent metaanalysis of human studies concluded that neither standard nor high volume haemofiltration with conventional haemofilters are able to significantly remove cytokines (Atan et al. 2013b). Only two studies of HVHF (Sanchez-Izquierdo et al. 1997, Cole et al. 2001) were included in the analysis (Atan et al. 2013b). In addition to cytokines other inflammatory mediators like prostaglandins and leukotrienes (Yokoyama et al. 2009), complement factors (Cole et al. 2001) and myocardial depressant factors (Blake et al. 1996) can be removed by HVHF.

Adsorption of cytokines to the haemofilter is considered an important mechanism of cytokine removal by haemofiltration (Honore et al. 2013).

Membranes with higher adsorptive capacities are more effective in cytokine removal, despite their lower cytokine filtration rates (Hirayama et al. 2011). Polyacrylonitrile or polymethyl metacrylate (PMMC) membranes exhibit most adsorptive capacities (Honore et al. 2013). For polyarylethersulfone (PAES) filters, removal of small molecular-weight proteins with molecular mass up to 24 kDa via both filtration and adsorption has been shown (Ouseph et al. 2008).

Several hypotheses have been proposed to explain the possible haemodynamic effects of HVHDF. The "peak concentration hypothesis" suggests that haemofiltration, applied in the early phase of sepsis eliminates the peaks of both anti- and proinflammatory cytokines, thus restoring immunohomeostasis (Ronco et al, 2004). The hypothesis stresses the importance of applying haemofiltration early in the course of the disease. Another, the "active transportation between two asymmetric compartments" hypothesis (Honore et al. 2012a), combining the "threshold immunomodulation hypothesis" (Honore et al. 2004) and the "mediator delivery hypothesis" (Di Carlo et al. 2005) postulates, that removal of cytokines from blood leads to increased gradient and therefore increased removal from tissues, thus limiting the systemic inflammation at tissue level. In addition to passive transportation, HVHF induces increase in lymphatic flow due to high amounts of crystalloids used as replacement fluids, which leads to significant drag and displacement of the cytokines to blood compartment, making them available for extracorporeal removal. This hypothesis explains why numerous studies failed to show significant decrease in cytokine plasma concentrations, as cytokines from tissues replace those, removed from blood compartment. The "cytokinic" theory suggests that removing inflammatory mediators from plasma increases concentration gradient from plasma to infected tissues, resulting in leucocyte homing to the nidus of infection instead of passing into the blood, thus increasing bacterial clearance locally and limiting remote organ damage (Namas et al. 2012, Honore et al. 2012b).

2.1.4 Effect of high volume haemodiafiltration on tissue perfusion

The effect of HVHF on microcirculation and tissue perfusion is not clear. Increase of blood pressure, if achieved via vasoconstriction, may theoretically lead to impaired tissue perfusion. An animal study found HVHF to be effective in reversing sepsis-induced hypotension, but not disturbances in microvascular, metabolic, endothelial and lung function (Sykora et al. 2008). Some human studies have reported increase in cardiac index with isovolemic HVHDF (Honore et al. 2000, Tapia et al. 2012), while others have concluded that increase of blood pressure occurs due to increased vascular resistance rather than increased cardiac output (Cornejo et al. 2006). Based on serum lactate levels as surrogate markers of tissue perfusion, HVHF seems to improve tissue metabolism as reflected by decreased serum lactate levels (Honore et al. 2000,

Cole et al. 2001, Cornejo et al. 2006). Although lactate removal by RRT corresponds to lactate concentration in plasma times millilitres filtrate per minute, it is only a fraction of lactate production, which is about 14 mmol/kg/min (Bollmann et al. 2004). Thus, the decrease in lactate more likely reflects improved circulation (Oudemans-van Straaten et al. 2013). Sublingual microcirculation during HVHF has been studied in 12 patients in severe hyperdynamic septic shock and no deterioration of microcirculation has been found despite increase in systemic vascular resistance (Ruiz et al. 2010).

In summary, HVHF seems to have a blood pressure stabilizing effect with possible improvement of tissue perfusion, at least in patients with refractory septic shock. Whether the effect is due to removal of pro- or anti-inflammatory cytokines or other substances, remains unclear.

2.2 Pharmacokinetics and pharmacodynamics of β-lactam antibiotics in septic patients with acute kidney injury

Beta-lactams (penicillins, cephalosporins and carbapenems) are the most commonly prescribed family of antibiotics (Roberts et al. 2014b). Their broad spectrum of activity and wide therapeutic window allows them to be widely used as empiric therapy in intensive care units (Cotta et al. 2014). Carbapenems and β-lactam/β-lactamase inhibitor combinations (BLBLIs) are used to treat infections caused by bacteria, resistant to other antibiotics, such as ESBL producing enterobacteria or *Pseudomonas* (Harris et al. 2015). While it has been shown that optimal exposure to β-lactams has positive effect on clinical cure and/or mortality of patients with severe sepsis and septic shock (Li et al. 2007, Scaglione et al. 2009, Crandon et al. 2010, Roberts et al. 2010b, Muller et al. 2013, Roberts et al. 2014c), adequate exposure is difficult to achieve in this patient population due to changes in pharmacokinetics and high inter-patient variability. Several studies have found that commonly used dosing regimens result in inadequate plasma concentrations of β-lactam antibiotics in critically ill patients undergoing RRT (Seyler et al. 2011, Roberts DM et al. 2012, Roberts et al. 2014c). Fluid extravasation due to capillary leak and fluid resuscitation may lead to substantial increase in volume of distribution (Goncalves-Pereira et al. 2011, Sime et al. 2012), potentially causing inadequate plasma concentrations at the beginning of antibacterial therapy, especially of hydrophilic antibiotics like β-lactams (Goncalves-Pereira et al. 2011, Sime et al. 2012). Achieving adequate antibiotic concentrations at the site of infection might further be delayed due to disturbances of microcirculation leading to impaired tissue penetration of the antibiotics (Joukhadar et al. 2001, Roberts et al. 2009b). As β-lactams are predominantly renally eliminated, decrease in renal function occurring in septic patients with AKI leads to reduced drug clearance, while extracorporeal clearance is highly dependent on the method and dose of renal replacement

therapy applied (Jamal et al. 2015b). The extent of extracorporeal clearance is also dependent on the protein binding of the drug. As only the free fraction can be eliminated by RRT, antibiotics with low or moderate protein binding are more affected by extracorporeal clearance (Choi et al. 2009). According to general belief highly protein bound antibiotics are not eliminated by RRT, yet decreased protein binding due to critical illness hypoalbuminaemia may result in their substantial extracorporeal clearance and insufficient plasma concentrations (Ulldemolins et al. 2011,).

2.2.1 Pharmacokinetic/pharmacodynamics target of β-lactam antibiotics

Beta-lactams are time-dependent antibiotics and the PK parameter most correlated to the antibacterial efficacy is the time above minimal inhibitory concentration (T>MIC) during the dosing interval (Vogelman et al. 1988, Craig 2003). For bactericidal effect, 35–40% of time above MIC has commonly been reported as sufficient for carbapenems (Drusano et al. 2003, van Wart et al. 2009) and 40–50% of time over MIC for penicillins (Craig 1998). A recent prospective study of eight β-lactam antibiotics in 248 infected critically ill patients found, that positive clinical outcome, defined as completion of treatment course without change or addition of antibiotic therapy was associated with antibiotic exposure of more than 50%T>MIC (Roberts et al. 2014c). In febrile neutropenia patients with bacteraemia clinical response rate was 80%, when meropenem concentrations exceeded MIC for 75% of time (Ariano et al. 2005). Maintaining carbapenem concentrations at four to six times over MIC throughout the dosing interval has been shown to ensure better cell kill and resistance suppression in *in vitro* and *in vivo* experiments with different isogenic strains of *Pseudomonas aeruginosa* (Tam et al. 2005, Louie et al. 2010, Soon et al. 2013). Some analyses of clinical data have reached similar results (Tam et al. 2002, Li et al. 2007, Taccone et al. 2012), and some recent PK/PD studies in critically ill patients undergoing RRT have used the target of 100%T>4xMIC (Jamal et al. 2015a, Jamal et al. 2015c)

In summary, treating infections in critically ill patients with β-lactam antibiotics at least 50%T>MIC should be targeted.

2.2.2 Pharmacokinetics of doripenem during renal replacement therapy

Doripenem is a carbapenem antibiotic with molecular mass of about 420 Da, hydrophilic properties and protein binding of 8%. (European Medicines Agency 2014).

During RRT, the PK of doripenem has mostly been studied in patients on continuous renal replacement therapies (Table 2). Cirillo and colleagues (Cirillo et al. 2011) performed a single dose PK study in end-stage renal failure patients

on continuous veno-venous haemofiltration (CVVH) with substitution fluid flow rate of 2 L/h, and on continuous veno-venous haemodiafiltration (CVVHDF) with dialysate and substitution fluid flow rate of 1 L/h each, and found that 38% and 29% of the administered doripenem dose was removed by CVVH and CVVHDF, respectively. Total body clearance of doripenem was 4.9 L/h and 5.9 L/h for CVVH and CVVHDF, respectively. Based on the data from this study, Samtani and colleagues recommended doses of 250 mg every 12 hours for treating infections caused by susceptible bacteria in patients undergoing CVVHF and 250 mg – 500 mg every 12 hours in patients undergoing CVVHDF (Samtani et al. 2012). Hidaka and colleagues studied critically ill patients with severe renal impairment undergoing low dose CVVHDF with dialysate flow rate of 0.5 L/h and substitution fluid flow rate of 0.3 L/h and found doripenem total body clearance of 3.5 L/h (Hidaka et al. 2010). A very small study from the same centre showed increased total body clearance of doripenem to 7.1 L/h, when CVVHDF was performed with dialysate flow rate of 1.5 L/h and effluent rate of 0.9 L/h (Ohchi et al. 2011). Roberts and colleagues studied the PK of doripenem in critically ill patients undergoing CVVHDF with mean dialysate and prefilter substitution fluid flow rate of 1 L/h each, found total body clearance of doripenem of 4.5 L/h and recommended dosing of 500 mg every 8 hours for critically ill patients to cover bacteria with MIC up to 4 mg/L (Roberts et al. 2014d). Wieczorek and colleagues studied the PK of doripenem in septic shock patients during slow low efficiency haemodialysis (SLED) with dialysis fluid flow rate of 125 mL/min and concluded that dosing pattern proposed by the manufacturer can be used in patients receiving CRRT SLED without necessary modifications (Wieczorek et al. 2014). Unfortunately the authors did not report, which dose was studied or which recommendations of the manufacturer regarding the type of RRT and patient's glomerular filtration should be followed.

Doripenem PK has also been studied during intermittent haemodialysis with dialysis fluid flow rate of 500 mL/min, and significant removal of doripenem with total body clearance of 7.1 L/h during dialysis has been shown (Tanoue et al. 2011). The authors recommended doses 500 mg every 12 hours for the first day, followed by 500 mg every 24 hours for treating infections caused by *Pseudomonas aeruginosa* (Tanoue et al. 2011). Another study recommended also 500 mg every 24 hours for treatment of *Pseudomonas* infections in chronic renal failure patients undergoing intermittent haemodialysis, based on measured doripenem concentrations above 2 mg/L in all time points with this dosing, no detailed pharmacokinetic analysis was performed (Heil et al. 2011).

2.2.3 Pharmacokinetics of piperacillin during renal replacement therapy

Piperacillin is an ureidopenicillin with hydrophilic properties, molecular mass of about 520 Da and protein binding of 30%. (Pfizer Ltd. 2013).

Pharmacokinetics of piperacillin has been studied during intermittent as well as during continuous renal replacement therapies and the results of the studies vary a great deal (Table 2). Francke and colleagues found that approximately 48% of the administered dose of piperacillin was recovered in dialysate within four hours, when intermittent haemodialysis was conducted with dialysate flow rate of 600 mL/min and blood flow rate of 250–300 mL/min (Francke et al. 1979). Similar haemodialysis clearance of piperacillin was described by Heim-Duthoy and colleagues (Heim-Duthoy et al. 1986). In contrast with these results, another study found that only 10% of the administered dose was removed during 4-hour haemodialysis session (Giron et al. 1981). Results of the early studies of continuous renal replacement therapies are similarly controversial. While Capellier and colleagues found no significant elimination of piperacillin with continuous veno-venous haemofiltration with mean ultrafiltrate flow rate of 0.8 L/h (Capellier et al. 1998), other studies have shown extracorporeal clearance exceeding 25% with continuous arterio-venous (Keller et al. 1995) or veno-venous hemofiltration (van der Werf et al. 1997, Joos et al. 1996, Arzuaga et al. 2005), haemodialysis (Mueller et al. 2002) and haemodiafiltration (Valtonen et al. 2001). These studies used conventional doses of continuous renal replacement therapy with ultrafiltrate flow rates of 0.8–1.5 L/h and dialysis flow rates of 1–2 L/h. Piperacillin clearance was shown to be dependent on ultrafiltration flow rate (van der Werf et al. 1997) and dialysis flow rate (Valtonen et al. 2001). Adding dialysis to filtration was shown to increase piperacillin clearance (Valtonen et al. 2001), while CVVHD was as efficient in removal of piperacillin as CVVHDF (Bauer et al. 2012). More recent studies have shown total piperacillin clearance of 3.9 L/h (Bauer et al. 2012) to 5.1 L/h (Varghese et al. 2014), depending on the intensity of RRT and on the patient's residual renal function (Arzuaga et al. 2005, Asin-Prieto et al. 2013). A recent regression analysis of published PK data also found effluent rate to be the main predictor of piperacillin clearance in critically ill patients undergoing continuous RRT (Jamal et al. 2014b). The most commonly recommended dose of piperacillin for these patients during continuous RRT is 4 g every 8 hours (Valtonen et al. 2001, Asin-Prieto et al. 2013, Varghese et al. 2014), while increasing the dose to 4 g every 4 hours is recommended for patients with normal renal function when they are treated with continuous RRT (Asin-Prieto et al. 2013).

Most studies have found significant interindividual variability in piperacillin concentrations (Francke et el. 1979, van der Werf et al. 1997, Giron et al. 1981, Mueller et al. 2002, Arzuaga et al. 2005, Seyler et al. 2011, Bauer et al. 2012, Asin-Prieto et al. 2013, Varghese et al. 2014).

Table 2. Pharmacokinetic studies of doripenem and piperacillin in patients receiving different types of renal replacement therapy

Author	Type of RRT/ Type (No.) of patients	RRT settings		Dose (mg); interval	PK parameters		
		Qb (mL/min)	Qe (mL/min)		V_d (L)	CL_T (L/h)	CL_{RRT} (L/h)
Doripenem							
Hidaka 2010	CVVHDF/ AKI (6)	100	13.3	250; Q12h or 24h	33.0 (15.8)	3.5 (0.8)	0.8 (0.1)
Ohchi 2011	CVVHDF/ AKI (2)	100	40	250 SD	NA	7.1	2.5
Cirillo 2011	CHF/ESRD (6)	125	33.3	500 SD	28.2 (14.1)	4.94 (2.13)	1.99 (0.03)
	CVVHDF/ ESRD (5)	125	33.3	500 SD	29.6 (6.4)	5.98 (1.19)	1.96 (0.05)
Tanoue 2011	HD/ ESRD (6)	150–200	500	500 SD	21.8 (6.0)	7.1 (1.2)	NR
Roberts 2014d	CVVHDF/ AKI (12)	200	33.3	500; Q8h	38.0	4.46	1.34
Piperacillin							
Francke 1979	HD/ ESRD (7)	300	600	1000 SD	10.4	5.9	NA
Giron 1981	HD/ ESRD (5)	NA	NA	1000 SD	26.7 (16.7)	3.3 (0.8)	0.5 (0.3)
Heim-Duthoy 1986	HD/ESRD (12)	250–500	544.6 (73.8)	4000; Q12h	18.2 (4.9)	5.5 (1.8)	NR
Keller 1995	CAVHD/ AKI (12)	NA	20.4	4000; SD	25.8 (3.8)	2.8 (0.7)	0.7 (0.06)
Joos 1996	CVVHF/AKI (8)	100	13.2	1000–4000; Q4–12h	NA	3.4	0.6
Van der Werf 1997	CVVHF/AKI (9)	NA	25.9	4000; Q8h	25.7	2.5 (1.4)	NA
Capellier 1998	CVVHF/AKI (6)	150	14.0 (1.0)	4000; first dose)	35.5 (17.8)	4.8 (1.5)	NA
	CVVHF/AKI (4)	150	10.8 (0.8	4000; Q8h	9.7 (4.8)	1.4 (0.8)	NA
Valtonen 2001	CVVHF/AKI (6)	100	13.3	4000; Q12h	NA	3.9 (1.2)	NA
	CVVHDF/AKI (6)	100	30	4000; Q12h	NA	5.1 (1.7)	NA
	CVVHDF/AKI (6)	100	46.7	4000; Q12h	NA	5.5 (2.1)	NA
Mueller 2002	CVVHD/AKI (8)	150	25	2000–4000; Q8–24h	20.8	2.8	1.3
Arzuaga 2005	CVVHF/AKI, CL_{CR} < 10 mL/min (4)	150–220	27.1 (7.8)	4000; Q6–8h	21.0 (11.7)	3.0 (3.1)	0.7 (0.4)
	CVVHF/AKI, CL_{CR} 10–50 mL/min (5)	150–220	30.3 (4.3)	4000; Q6–8h	26.8 (19.8)	5.4 (1.8)	0.7 (0.8)
	CVVHF/AKI, CL_{CR} > 50 mL/min (5)	150–220	20.0 (7.5)	4000; Q6–8h	44.9 (20.4)	15.9 (9.1)	0.3 (0.2)
Seyler 2011	CVVHF/CVVHDF/AKI (8)	NR	25.6 (52.5)	4000; Q6h	30.8 [15.4–120.4]	4.8 [1.1 – 26.3]	NR
Bauer 2012	CVVHD or CVVHDF/AKI or ESRD (42)	NR	40.7 (25.0)	2000–3000; Q6–12h	38.2 (26.5)	4.7 (3.7)	1.9 (0.9)
Asin-Prieto 2013	CVVHF/AKI 16	140–230	26.9 (7.1)	4000; Q4–8h	42.3	6.6	0.6 (0.5)
Varghese 2014	CVVHDF/AKI (10)	200–250	50–65	4000; Q8h	34.9 [24.1–40.7]	5.1 [4.2–6.2]	2.5 [2.3–3.1]

AKI – acute kidney injury, CAVHD – continuous arterio-venous haemodialysis, CL_{CR} – creatinine clearance, CL_{RRT} – clearance by renal replacement therapy, CL_T – total body clearance, CVVHD – continuous veno-venous haemodialysis, CVVHDF – continuous veno-venous haemodiafiltration, CVVHF – continuous veno-venous haemofiltration, ESRD – end-stage renal disease, HD – haemodialysis, NA – not available, Qe – effluent flow rate, Qb – blood flow rate, RRT – renal replacement therapy, SD – single dose, V_D – volume of distribution. Data given in mean (SD) or median [interquartile range]

In summary, pharmacokinetics of doripenem and piperacillin/tazobactam has not been studied during HVHDF. Different methods of RRT result in different clearance of both antibiotics. While effluent rate seems to be the RRT parameter most correlated to extracorporeal clearance of β-lactams (Jamal et al. 2014b), systemic clearance of these antibiotics may not be consistently RRT dose dependent (Roberts DM et al. 2015). No dosing recommendations for HVHDF can be derived from the existing PK data.

2.2.4 Pharmacokinetics of tazobactam during renal replacement therapy

Tazobactam is a β-lactamase inhibitor, added to β-lactam antibiotics to extend their spectrum of activity and retain the activity of the β-lactam antibiotic despite the effect of hydrolysing β-lactamase enzymes, although β-lactamase inhibitors have little antibiotic activity of their own (Harris et al. 2015). It is a hydrophilic molecule with molecular mass of about 300 Da. (European Chemical Agency 2014). In healthy volunteers the PK of tazobactam is similar to piperacillin with similar terminal elimination half-life, protein binding, volume of distribution and predominantly renal elimination (Occhipinti et al. 1997).

During continuous renal replacement therapy total body clearance of tazobactam from 2.4 L/h (CVVHF, Valtonen et al. 2001) to 3.8 L/h (CVVHDF, effluent rates of 3–3.9 L/h, Varghese et al. 2014) has been described. Some studies (van der Werf et al. 1997, Mueller et al. 2002, Asin-Prieto et al. 2013) have shown relative accumulation of tazobactam and suggested increasing piperacillin dose relative to that of tazobactam to avoid tazobactam cumulation.

2.2.5 Administration via bolus or extended infusion

Several studies and modelling of PK data of β-lactam antibiotics show better PK/PD target achievement in critically ill patients when given as extended or continuous antibiotic infusion instead of intermittent bolus (Li et al. 2005, Bhavnani et al. 2005, Langgartner et al. 2007, Roberts et al. 2009a, Roberts et al. 2010a, Samtani et al. 2012, Felton et al. 2012, Dulhunty et al. 2013, Asin-Prieto et al. 2014, De Waele et al. 2014). Jamal et al. compared continuous and intermittent bolus administration of piperacillin/tazobactam in critically ill patients undergoing continuous haemofiltration and found better PK/PD target attainment with continuous infusion (Jamal et al. 2015c). A study of meropenem in similar settings did not find any differences in PK/PD target attainment (Jamal et al. 2015a). Meropenem doses used were as recommended for normal renal function, while piperacillin/tazobactam was used in reduced doses (Jamal et al. 2015a, Jamal et al. 2015c). In both studies both groups received a bolus loading dose. Meropenem doses used were the recommended doses for normal renal function, while piperacillin/tazobactam was used in reduced doses (Jamal et al. 2015a, Jamal et al. 2015c). In terms of mortality

and/or clinical cure the evidence of benefit from extended or continuous infusion of β-lactam antibiotics is less convincing (Lorente et al. 2009, Hsaiky et al. 2013, Lodise et al. 2007). Some randomized controlled trials have showed equivalence of continuous β-lactam infusion compared to bolus administration (Lau et al. 2006, Georges et al. 2005, Hanes et al. 2000, Nicolau et al. 2001). In a randomised open-label study continuous infusion of ceftriaxone suggested clinical and bacteriological benefit (Roberts et al. 2007). A multicentre double-blind randomized controlled trial comparing intermittent dosing of piperacillin/ tazobactam, meropenem and ticarcillin/clavulanate to continuous administration found better clinical cure in the continuous administration group (Dulhunty et al. 2013a). There was no difference in ICU-free days or mortality, but the study was not powered to evaluate effect on survival (Dulhunty et al. 2013a). Two meta-analyses of mostly non-randomised studies found clinical benefit or lower mortality for extended or continuous infusion of β-lactam antibiotics (Falagas et al. 2013, Teo et al. 2014). In the meta-analysis by Falagas and colleagues, the effect on mortality was present in carbapenems and piperacillin/tazobactam analysed together and piperacillin/tazobactam alone, but not for carbapenems alone (Falagas et al. 2013). A Cochrane database systematic review on the other hand concluded that continuous infusion of antibiotics has no benefit over standard intermittent infusion in terms of mortality, infection recurrence, clinical cure, super-infection or safety (Shiu et al. 2013). A recent meta-analysis, comparing extended and continuous infusion of piperacillin/ tazobactam to intermittent bolus administration and including 5 randomised controlled trials and 9 observational studies, found higher clinical cure rate and lower mortality rate with extended or continuous infusion (Yang et al. 2015). A very recent large randomized controlled trial comparing the effect of intermittent vs continuous administration of three β-lactam antibiotics (ticarcillin/clavulanate, piperacillin/tazobactam and meropenem) in 432 patients with severe sepsis found no difference in ICU-free days at day 28 as the primary outcome (Dulhunty et al. 2015).

2.3 Summary of literature

High volume haemodiafiltration as an extracorporeal blood purification method might be useful in the treatment of patients with refractory septic shock in order to control excessive immunological reaction and achieve haemodynamic stabilisation, when conventional methods fail. As removal of antibiotics is dependent of the dose and method of RRT, potentially excessive removal of antibiotics during HVHDF may lead to low plasma concentrations, ineffective antibacterial therapy, worse clinical outcome and potential escalation of antibiotic resistance. On the other hand, increasing doses of antibiotics empirically may lead to toxicity. No pharmacokinetic data of β-lactam antibiotics during HVHDF are available and no dosing recommendations for HVHDF can be derived from the existing PK data.

3. AIMS OF THE STUDY

The general aim of the thesis was to describe the influence of high volume haemodiafiltration on the pharmacokinetics of two beta-lactam antibiotics and systemic inflammatory reaction in patients with severe sepsis and septic shock. We hypothesized that HVHDF markedly changes the pharmacokinetic profile of doripenem and piperacillin/tazobactam, and is associated with beneficial modifications of whole body inflammatory response in patients with severe sepsis and septic shock

The specific aims were as follows:

1. To describe the pharmacokinetics of doripenem during HVHDF in patients with severe sepsis and septic shock with acute kidney injury in order to define optimal dosing for this method of RRT in this patient group.
2. To describe the pharmacokinetics of piperacillin and tazobactam during HVHDF in patients with severe sepsis and septic shock with acute kidney injury in order to define optimal dosing for this method of RRT in this patient group.
3. To find out if HVHDF influences cytokines profile and/or has effect upon central haemodynamics or sublingual microcirculation in critically ill severe sepsis and septic shock patients.

4. PATIENTS AND METHODS

The thesis is based on a clinical study with three substudies conducted in the general intensive care unit (ICU) of Tartu University Hospital from September 1, 2011 till June 25, 2014 (Table 3)

Table 3. Description of studies and analyses of the thesis.

Substudy	Timing	Population	Primary aim	Publication
Pharmacokinetics of doripenem during HVHDF	01.09.2011 – 31.08.2012	9 patients with septic shock	Population PK of doripenem. To find the dose for extended HVHDF	I
Pharmacokinetics of piperacillin/tasobactam during HVHDF	01.09.2012 – 25.06.2014	10 patients with severe sepsis and septic shock	Population PK of piperacillin and tazobactam To find the dose for extended HVHDF	II
Modification of inflammatory response by HVHDF in patients with severe sepsis and septic shock.	01.09.2011 – 25.06.2014	19 patients with severe sepsis and septic shock	To find out if HVHDF influences cytokines profile and/or sublingual microcirculation septic shock patients.	III

4.1 Ethical considerations

The study was approved by the Research Ethics Committee of the University of Tartu and by State Agency of Medicines of Estonia (EU Clinical Trials Register No. 2011-000644-16). Informed consent from next of kin was obtained for all patients prior to study inclusion. The patient's informed consent was obtained retrospectively, if he/she recovered sufficiently.

The blood loss from blood sampling was not clinically significant as the blood volume removed was 60 mL, which is no more than 1.5% of the presumed circulating blood volume.

4.2 Study patients

Adult patients were eligible for the study, if they had severe sepsis or septic shock as defined by the ACCP/SCC Consensus Conference as follows: (1) severe sepsis: systemic response to infection manifested by two or more of the following conditions as a result of infection: temperature >38°C or <36°C,

heart rate >90 beats per minute, respiratory rate >20 breaths per minute or PaCO<32 mm Hg, leucocytosis or leucopenia, and signs of organ dysfunction, hypoperfusion or hypotension; (2) septic shock: sepsis-induced hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities (Bone et al. 1992). The patients had to have acute kidney injury (AKI) deemed by the treating clinician to require HVHDF, based on the presence of at least one of the following criteria: (1) oliguria (urine output <100 ml in a 6-hour period), unresponsive to fluid therapy, (2) serum potassium concentration exceeding 6.5 mEq/L, (3) severe acidemia (pH <7.2), (4) plasma urea nitrogen level above 70 mg/dL, (5) serum creatinine concentration above 3.4 mg/dL, or (6) presence of clinically significant organ oedema (e.g., pulmonary oedema), and an arterial line *in situ*. Patients with known hypersensitivity to carbapenems, penicillins or other beta-lactams, pregnancy, and life expectancy of less than 8 hours were excluded.

4.3 High volume haemodiafiltration

Extended HVHDF in the ultracontrol predilution mode with AK 200 ULTRA S (Gambro, Lund, Sweden) as described by Kron et al (Kron 2011) was applied. At the beginning of treatment this mode titrates filtration volume to the maximal possible by gradually increasing transmembrane pressure by 25 mmHg every 30 seconds until maximal filtration rate is achieved. Subsequently, this pressure is maintained at maximum effectiveness by testing and adjusting once every hour. HVHDF was performed with capillary dialyzer Polyflux 210H (Gambro Dialysatoren, Hechingen, Germany) with surface area 2.1 m^2 and ultrafiltration coefficient 85 ml/h/mmHg. The procedure was performed via 3-lumen central venous catheter with blood flow rate of 200 mL/min and fluid flow rate of 500–650 mL/min. The substitution fluid is taken from the dialysis fluid thus reducing the dialysis fluid flow rate. The prescribed duration of the HVHDF was 10 hours.

4.4 Patient monitoring

All patients were treated in third level intensive care unit and thus thoroughly monitored on clinical grounds. Clinical blood tests (red blood cells, white blood cells, haemoglobin, haematocrit and platelet count), serum biochemistry, arterial blood gases and lactate were monitored according to clinical routine of the ICU. Serum electrolytes (sodium, calcium, and phosphate), creatinine and urea were measured before the HVHDF procedure, and within 24 hours after it. Serum potassium, glucose and activated partial thromboplastin time (APTT) were monitored every four hours during the HVHDF procedure. Body temperature was continuously monitored during the haemodiafiltration procedure and external warming applied, if necessary.

All patients were monitored for adverse events for at least 7 days after study drug administration. Monitoring included clinical evaluation for occurrence of allergic reactions and seizures, laboratory and vital parameters and microbiological cultures. Concomitant medications were recorded during the whole ICU stay.

4.5 Substudy I. Pharmacokinetics of doripenem during high volume haemodiafiltration

4.5.1 Study drug administration and sample collection

A single dose of 500 mg of doripenem (Doribax, Janssen-Cilag International N.V) in 50 mL of 0.9% sodium chloride was administered in addition to the ongoing antibacterial therapy as a 1 hour intravenous infusion. The dose was administered one hour after the start of HVHDF via a central venous cannula, which was not used for hemodiafiltration.

Blood samples of 4 mL were collected before and immediately after the end of doripenem infusion and 90, 120, 150, 180, 210, 240, 300, 360, 420 and 480 min after the start of drug administration. Blood samples were drawn to lithium heparin containing tubes from pre-existing arterial cannula. Blood samples were centrifuged immediately (10 min at 3500 rpm), plasma was separated, stored at –20ºC for a maximum of 12 hours and then transferred to –80ºC until analysed within 12 months.

Doripenem plasma concentrations were measured with ultrahigh performance liquid chromatography-double mass spectrometry as detailed in paper I (pp. 439 – 440).

4.5.2 Non-compartmental pharmacokinetic analysis

Based on the observed plasma levels of total concentrations, the half-life ($T_{1/2}$) and the elimination rate constant (Kel) of doripenem during HVHDF were derived using noncompartmental analysis. Plasma concentrations and PK parameters of doripenem during HVHDF were summarized using descriptive statistics. All calculations were performed using the WinNonlin Phoenix version 6.5.1 (Pharsight Corporation, CA).

4.5.3 Population pharmacokinetic analysis

PK data were analysed by non-linear mixed effect modelling (NLME) using NONMEM 7.3.0 by Joseph Standing (University College of London). One-, two- and three-compartment linear models were tested. Inter-individual variability was described using an exponential error model and residual unexplained variability with a proportional error model. The selection of the

population model and residual error model was driven by goodness-of fit plots and an improvement in the objective function value (OFV). The potential effect of covariates on PK parameters was evaluated if a correlation was biologically plausible or by the visual inspection of box and scatter plots of individual estimates derived from the final model and inter-individual variability (ETAs) compared against covariates. Covariates that reduced the OFV by at least 6.635 points ($p<0.01$) were considered statistically significant and included in the subsequent covariate analysis.

4.5.4 Pharmacodynamic target attainment

The parameter estimates of the final PK model were used as inputs within 1000 Monte Carlo simulations to assess the PD target attainment ability of doripenem on HVHDF. Fraction of time over minimal inhibitory concentration (fT>MIC) for 50% of dosing interval was selected as PD target (Roberts et al. 2014c). Mean serum protein binding of doripenem is around 8% (European Medicines Agency) and this was accounted for in simulations.

Since in vitro data have shown that Gram negative resistance to doripenem can develop over the first 48 hours of treatment (Bowker et al. 2012), the target MIC was allowed to double over this period using a linear model. Continuous HVHDF was assumed and the probability of target attainment (PTA) after the first dose and at 48 hours was calculated for dosing regimens of 500mg and 1000 mg every 6, 8, 12 and 24 hours for the European Committee of Anti-microbial Susceptibility Testing (European Committee on Antimicrobial Susceptibility Testing 2014) defined MIC susceptibility and resistance break-points of doripenem for *Enterobacteriaceae* and *Pseudomonas* of ≤1 and >2 μg/mL. In 2013, when the analysis was conducted, the EUCAST resistance breakpoint for both *Pseudomonas* and *Enterobacteriaceae* was <4 mg/L (European Committee on Antimicrobial Susceptibility Testing 2013), so the simulations were conducted also for MIC of 4 μg/mL.

4.6 Substudy II. Pharmacokinetics of piperacillin/ tazobactam during high volume haemodiafiltration

4.6.1 Study drug administration and sample collection

A single dose of 4000 mg of piperacillin and 500 mg of tazobactam in fixed commercially available combination in 50 mL of 0.9% sodium chloride was administered in addition to the ongoing antibacterial therapy as a 30 minute intravenous infusion. The dose was administered one hour after the start of HVHDF via a central venous cannula, different from that used for haemo-diafiltration.

Blood samples of 4 mL were collected before and immediately after the end of piperacillin/tazobactam infusion and 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min after the start of drug administration. In two patients samples were also collected 12 h and 24 h after the study drug administration, when the patients were off RRT. Blood samples were drawn to lithium heparin containing tubes from arterial cannula. Blood samples were centrifuged immediately (10 min at 3500 rpm), plasma was separated, stored at –20°C for a maximum of 12 hours and then transferred to –80°C until analysed within 12 months.

Piperacillin and tazobactam plasma concentrations were measured with ultrahigh performance liquid chromatography-double mass spectrometry as detailed in paper II (pp. 5–7).

4.6.2 Non-compartmental pharmacokinetic analysis

Based on the observed plasma levels of total concentrations, the half-life ($T_{1/2}$) and the elimination rate constant (Kel) of piperacillin and tazobactam during HVHDF were derived using noncompartmental analysis. Plasma concentrations and PK parameters of piperacillin and tazobactam during HVHDF were summarized using descriptive statistics. All calculations were performed using the WinNonlin Phoenix version 6.5.1 (Pharsight Corporation, CA).

4.6.3 Population pharmacokinetic analysis

Piperacillin and tazobactam were modelled separately by Tõnis Tasa (Faculty of Mathematics and Computer Science, University of Tartu). Plasma concentrations observed during HVHDF were fitted to a 1- and 2-compartment model with zero-order input and first-order elimination. Non-linear mixed effects modelling approach was used and model training was based on iterative improvements of log-likelihood values. Model discrimination was accomplished by visual inspection of the predicted versus observed concentration data, visual inspection of the distribution of the standardized residuals over fitted values and over time, log-likelihood values, Akaike information criteria and sums of the squared weighted residuals. Visual predictive checks were created by evaluation of 1000 concentration-time profiles for each of the two models. From these the median concentration profile with 90% confidence intervals were deduced and overlaid on the observed concentration measurements.

Random effects without an underlying covariance structure of diagonal structure were modelled for all fixed effects and log-normal distribution was assumed.

For piperacillin, residual variability was modelled using a combined additive power variance structure where variance equals to a constant plus power of the variance covariate where the residuals are distributed as $N\sim(0,\sigma^2*(\delta_1+|\eta|^{\delta 2})^2)$ where η is the fitted value covariate, σ is standard deviation of the residuals, δ_1 is the constant parameter and δ_2 the power parameter.

For tazobactam, residual variability was modelled using an exponential model where the residuals follow normal distribution of the form N(0, $\sigma^2 * \exp(2*\delta*\eta)$, where η is the fitted value covariate, σ is standard deviation of the residuals and δ is the exponential parameter.

4.6.4 Pharmacodynamic target attainment

The final piperacillin population PK model was used in a 1000-patient Monte Carlo simulation (MCS) to generate concentration-time profiles for various dosing regimens and lengths of infusions during HVHDF at steady-state. The free drug time over minimal inhibitory concentration (fT>MIC) at steady state was calculated for piperacillin 4g administered every 6 and 8 hours as 0.5h and 4h infusion. The EUCAST defined susceptibility and resistance breakpoint MIC-s of ≤8 and >16 mg/L for *Enterobacteriaceae*, ≤16 and >16 mg/L for *Pseudomonas* (EUCAST 2015) and serum protein binding of 30% (Roberts et al. 2009a) for piperacillin were used in the calculations. For all dosing regimens probabilities of target attainment were found for targets of 50% and 100% of fT>MIC with bootstrapped 90% confidence intervals.

The final tazobactam model was used in 1000-patient MCS to simulate concentration-time profiles during HVHDF for dosing regimens of 500 mg as 0.5h infusion every 6 and 8 hours. Population PK analysis and MCS were performed using base R (version 2.15.2 (2012-10-26), The R Foundation for Statistical Computing) and package "nlme" (Pinheiro et al. 2014).

4.7 Substudy III. Modification of inflammatory response by high volume haemodiafiltration in patients with severe sepsis and septic shock

4.7.1 Blood sample collection and handling

Blood samples were taken from a pre-existing arterial cannula immediately before the start and after the end of HVHDF session in the study, immediately centrifuged and stored at –20°C for a maximum of 12 hours and then transferred to –80ºC until analysed.

The following cytokines and growth factors: IL-1, IL-2, IL-6, IL-8, TNF-α, IF-γ, MCP-1, IL-4, IL-10, EGF and VEGF were measured in sera with the Evidence Investigator Cytokine & Growth factors high-sensitivity array (CTK HS Cat. No. EV 3623; RANDOX Laboratories Ltd. Crumlin, UK) according to the manufacturer's protocol in the Institute of Biochemistry of the University of Tartu. Assay sensitivity varied from 0.12 pg/L to 2.12 pg/L depending on specific marker analyte. The reproducibility of the assay for individual cytokine was determined using the quality controls provided with the kit.

4.7.2 Videomicroscopy

Sublingual microcirculation was assessed before and after HVHDF by a side-stream dark-field (SDF) imaging device (Microscan; Microvision Medical, Amsterdam, The Netherlands). In total, at least nine videos were taken at each time point, of which five best were analysed by two observers.

Microcirculatory videos of all patients were collected and thereafter analysed with the aid of specialized software (Automated Vascular Analysis 3.02; Academic Medical Centre, University of Amsterdam, The Netherlands) by 2 separate investigators unaware of the study protocol.

4.7.3 Statistical analysis

All except interobserver variability calculations were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 20.0, Somers, NY, USA) software. Normal distribution of data was confirmed by visual inspection of result histograms. Data with normal distribution are presented as mean (SD – standard deviation) and data not normally distributed as median (IQR – interquartile range). Paired t-test for normally distributed data, and Wilcoxon matched pairs test for not normally distributed data were used to compare prediafiltration values against postdiafiltration values. Differences were considered significant at $p < 0.05$.

Interobserver variability was calculated separately for each parameter through the Bland-Altman analysis for assessing agreement between two opinions (Bland et al. 1986) using StatsDirect 2.7.9 software (StatsDirect Ltd, Cheshire, UK).

5. RESULTS

5.1 Patients

Of 36 screened patients, 19 were enrolled in the study: nine in substudy I and 10 in substudy II, while all the patients participated in substudy III. The reasons for exclusion were absence of informed consent from next of kin (11 patients), life expectancy of < 8 hours (5 patients), and age <18 years (1 patient). The demographics and clinical characteristics of the patients at baseline are shown in Table 4. Eighteen patients were mechanically ventilated and all except one received norepinephrine at the start of HVHDF. In addition six patients received dopamine, dobutamine or milrinone. Bedside nurses maintained the mean arterial pressure (MAP) at the target range, defined by the treating physician (70–80 mmHg) by adjusting the dose of norepinephrine infusion. The focus of infection was pneumonia in 6 cases, intraabdominal (peritonitis, cholangitis, infected pancreatic necrosis) in 10 cases and soft tissue in 3 cases. The median (range) time from ICU admission to the study inclusion was 45 (6–361) hours. Seventeen patients had received renal replacement therapy before the studied HVHDF. The median (IQR) fluid balance during the studied HVHDF was –409 (–1405–25.75) mL. Sixteen patients were discharged from ICU alive, three died in the ICU: one within 48 hours after study inclusion due to refractory septic shock and two more than two weeks later due to multiple organ failure. The median (IQR) ICU stay of the patients was 23 (9–44) days.

The patient's estimated glomerular filtration rate (eGFR) was calculated according to the MDRD formula (Levey et al. 2006).

Table 4. Demographic and clinical characteristics of study patients at baseline.

	Median (IQR)
Age (years)	65 (56–72)
Gender (male/female)	12/7
Weight (kg)	80 (70–100)
APACHE II	19 (16–20)
SOFA	10 (7–11)
C-reactive protein (mg/L)	252 (121–316)
Creatinine before inclusion (μmol/L)	164 (127–348)
eGFR (mL/min/1.75m^2)	18 (11–28.5)
Lactate before inclusion (mmol/L)	1.5 (1.2 – 3.0)
ICU cumulative fluid balance (mL)	3328 (–702–5381)

APACHE II – Acute Physiology and Chronic Health Evaluation score II, eGFR – estimated glomerular filtration rate, ICU – intensive care unit, SOFA – Sequential Organ Failure Score

5.2 High volume haemodiafiltration

The studied HVHDF was performed for 10 hours in 17 patients. In two patients of the piperacillin/tazobactam substudy group the HVHDF was terminated after 4 hours due to filter clotting. One patient in the doripenem substudy group had a 1-hour and one patient in the piperacillin/tazobactam substudy group had a 2-hour break in HVHDF due to filter clotting. The mean (SD) time of HVHDF was 9.4 (1.8) hours. The median convective volume was 11.5 L/h (IQR 8.8 – 15.9) [123 mL/kg/h (IQR 100–211 mL/kg/h)]. Anticoagulation was performed with unfractionated heparin or heparin/protamine as clinically indicated.

5.3 Adverse events

One patient developed atrial fibrillation during the HVHDF and one developed severe hypotension at the beginning of the procedure, stabilized with infusion therapy and temporary increase in norepinephrine dose. No other adverse events including hypothermia, severe hypophosphataemia or hypokalaemia were observed.

No adverse events or changes in laboratory parameters were documented after doripenem or piperacillin/tazobactam administration (Table 5).

Table 5. Laboratory parameters of study patients before and after HVHDF.

	Pre HVHDF	Post HVHDF	p
Hb (g/L)	101 (91–120)	104 (90–110)	NS
Hct	0.31 (0.27–0.38)	0.31 (0.27–0.33)	NS
WBC *10^9/L	11.4 (6.1–19.7)	12.3 (5.9–21.0)	NS
CRP	252 (121–316)	246 (119–313)	NS
Bilirubin	22.5 (14.5–47.75)	19.5 (12.0–42.25)	NS
ALT	29 (19–135)	29 (18–152)	NS
AST	42 (21–68)	39 (24–233)	NS
Albumin	28 (25.5–32.5)	27 (26–34)	NS

ALT – alanine aminotranferase, APAHE II – acute physiology and chronic health evaluation II score, AST – aspartate aminotransferase, CRP – C-reactive protein, Hb – haemoglobin, Hct – hematocrit, WBC – white blood cells

5.4 Substudy I. Pharmacokinetics of doripenem during high volume haemodiafiltration

5.4.1 Non-compartmental PK analysis

Nine subjects were included in the PK analysis. Doripenem concentrations were measurable 480 min post dose and exceeded the EUCAST susceptibility criteria MIC value of 1 mg/L for 8/9 subjects. Doripenem concentrations demonstrated high variability after a single 500 mg 1 hour infusion (Figure 2).

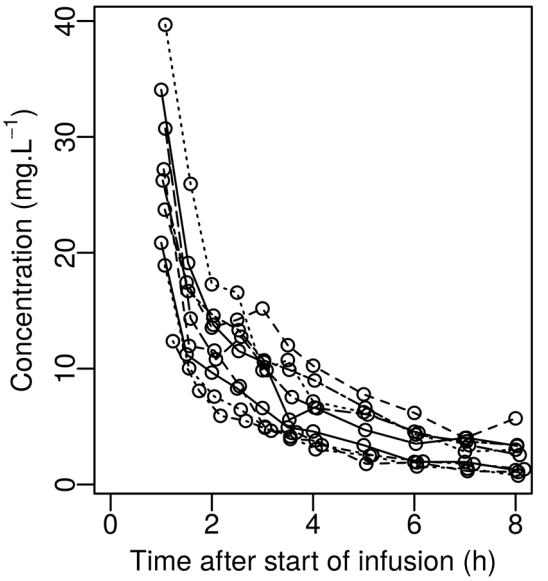

Figure 2. Plasma concentration-time profiles of doripenem

The PK parameters of doripenem in the study patients are shown in Table 6.

Table 6. Noncompartmental PK parameters of doripenem during HVHDF.

Patient	C_{max} (mg/L)	K_{el} (/h)	$T_{1/2}$ (h)	V_d (L)	AUC_τ (mg*h/L)	AUC_{inf} (mg*h/L)	CL (L/h)
ID-1	20.9	0.340	2.0	29.2	46.9	50.6	10.6
ID-2	27.2	0.398	1.7	22.9	48.2	51.0	10.3
ID-3	18.9	0.375	1.8	31.0	38.6	40.6	12.9
ID-4	12.4	0.258	2.7	54.6	32.9	38.0	15.0
ID-5	30.7	0.286	2.4	23.7	70.1	79.0	7.1
ID-6	26.2	0.263	2.6	25.3	75.0	87.9	6.6
ID-7	34.1	0.262	2.6	23.8	74.0	86.7	6.7
ID-8	23.7	0.212	3.3	30.5	83.8	110.7	5.9
ID-9	39.7	0.334	2.1	16.0	89.7	98.7	5.5
Mean (SD)	26.0 (8.2)	0.303 (0.06)	2.4 (0.5)	28.5 (10.8)	62.1 (20.7)	71.5 (26.9)	8.9 (3.4)

AUC_{inf} – area under the time-concentration curve extrapolated to infinity; AUC_τ – area under the time-concentration curve during the dosing interval; CL – total body clearance; C_{max} – maximal concentration; K_{el} – elimination rate constant; $T_{1/2}$ – terminal elimination half life ; V_d – volume of distribution

5.4.2 Population PK analysis

A 2-compartment model fitted the concentration–time data best. Goodness of fit plots and visual predictive check of the final model are shown in Figures 3 and 4. Inclusion of the tested covariates (serum creatinine, APACHE II, effluent rate, effluent volume and fluid removal volume) did not significantly improve the model fit and they were not retained in the final model. The PK parameter estimates of the final PK model are shown in Table 7.

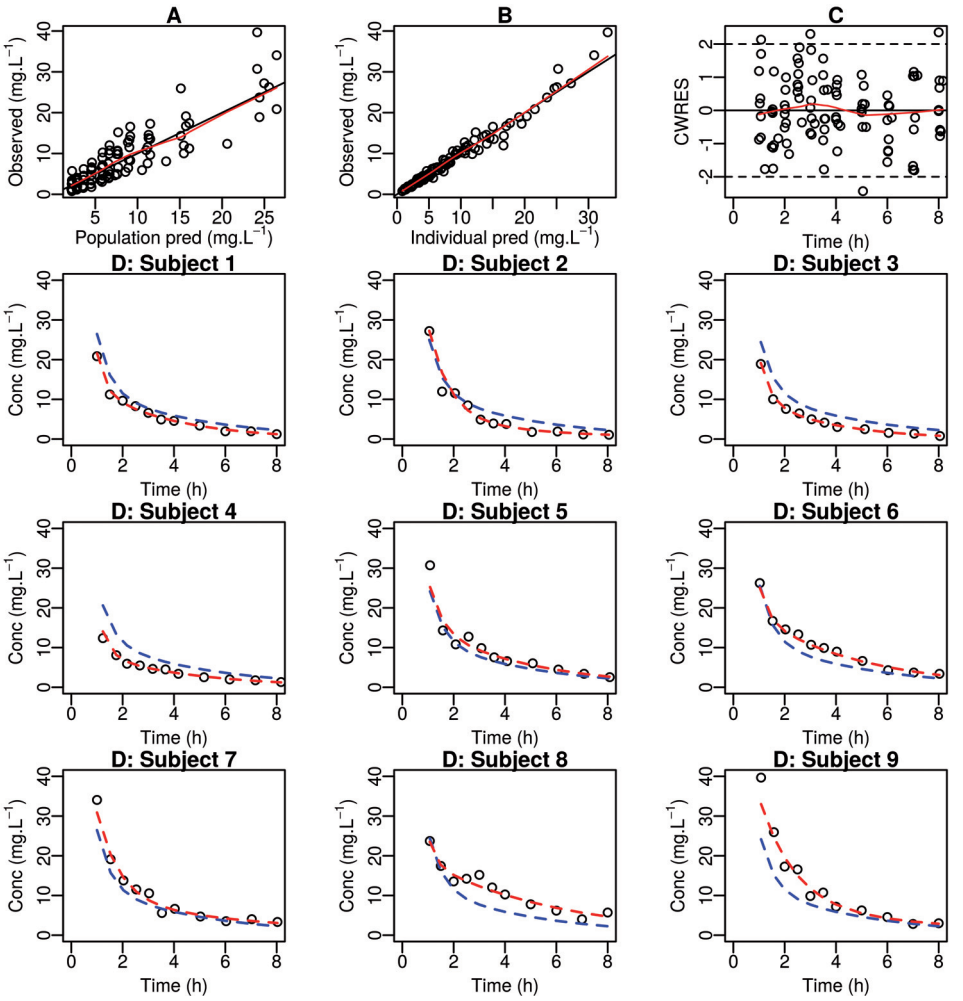

Figure 3. Goodness of fit plots from the final doripenem population PK model. A: Population predictions versus observations; B: Individual predictions versus observations; C: Conditional weighted residuals versus time; D: Individual plots with observations as open circles, population predictions as blue dashed line, individual predictions as red dashed line.

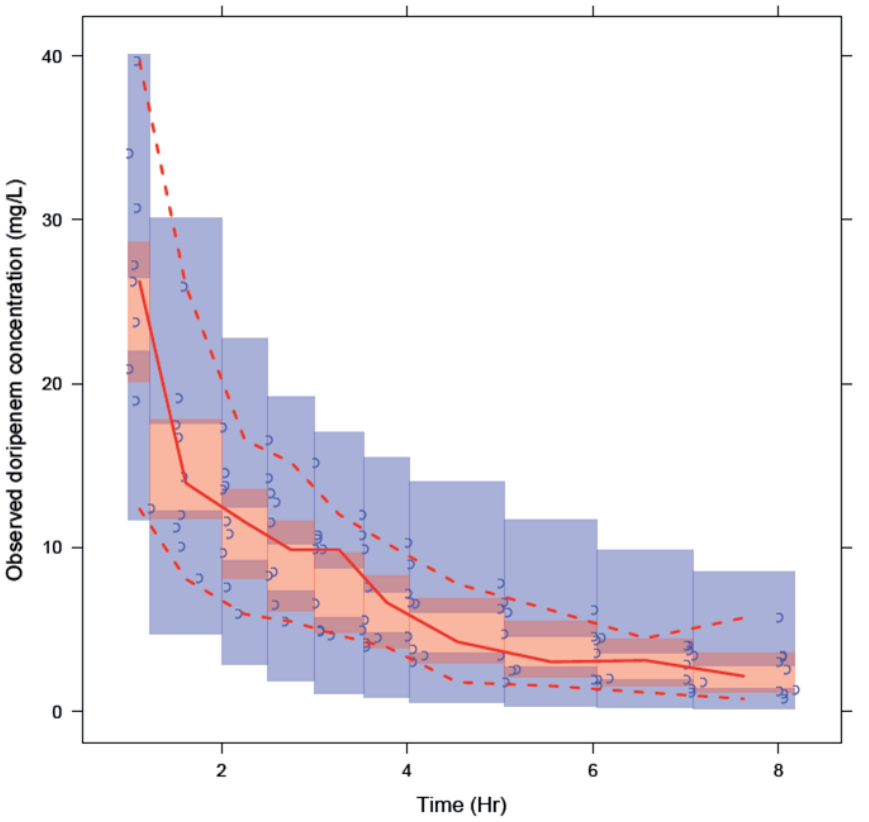

Figure 4. Visual predictive check of the final doripenem population model. The dashed bottom line marks the 5th percentile, the solid middle line marks the 50th percentile and the top dashed line marks the 95th percentile of model predicted data. The open circles represent the observed data, shaded areas are the corresponding 95% confidence intervals of model-simulated percentiles.

Table 7. Parameter estimates of the final doripenem population PK model.

Parameter	Estimate	IIV	RSE	Bootstrap CI		
CL (L/h)	6.82	33%	12%	6.80	6.95	7.19
V1 (L)	10.8	0%	7.6%	10.54	10.68	10.82
Q (L/h)	8.75	79%	30%	8.29	9.17	10.04
V2 (L)	12.1	25%	11%	12.01	12.8	13.64

CI – 95% confidence interval; CL– total doripenem clearance; IIV – inter-individual variability; Q – intercompartmental clearance; RSE – residual standard error; V1 – volume of distribution in the central compartment; V2 – volume of distribution in the peripheral compartment

5.4.3 Probability of target attainment

Figures 5 and 6 demonstrate the spread of the fT>MIC for the 1000 simulated patients for the tested dosing regimens after the first dose and 48 hours. The median probability of target attainment at 48 hours for 500 mg dosed every 6, 8, 12 and 24 hours were 99.9%, 98.8%, 85.7% and 19.9% respectively for an initial MIC of 1 μg/mL. For 1000 mg doripenem doses the median PTA values for initial MIC of 4 μg/mL at 48 hours were 97.2%, 88.5%, 49.8% and 1.8% respectively for dosing every 6, 8, 12 and 24 hours.

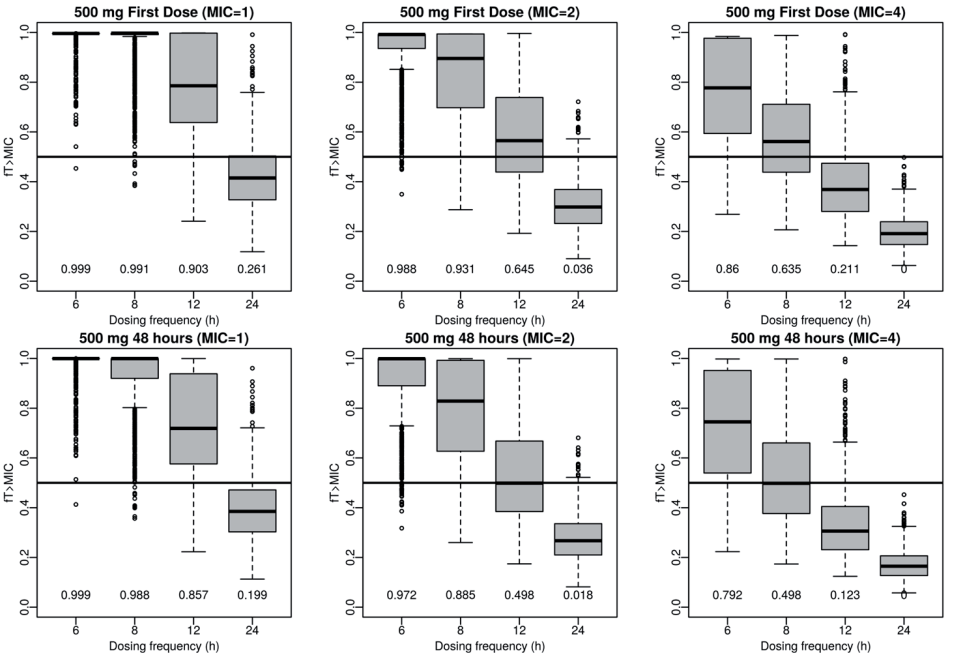

Figure 5. Box and whisker plots for fT>MIC of 1 μg/mL (left), 2 μg/mL (middle) and 4 μg/mL (right) for different dosing frequencies of 500 mg of doripenem after 1 dose and after 48 hours. These figures assume MIC doubles over the first 48 hours of treatment. Probability of target attainment given at the bottom of each plot with the target set at fT>MIC of 50%. The bottom and top of the box represent first and third quartile, respectively, the line inside the box represents median, the whiskers represent the box quartile +/– the interquartile range and the open circles represent outliers.

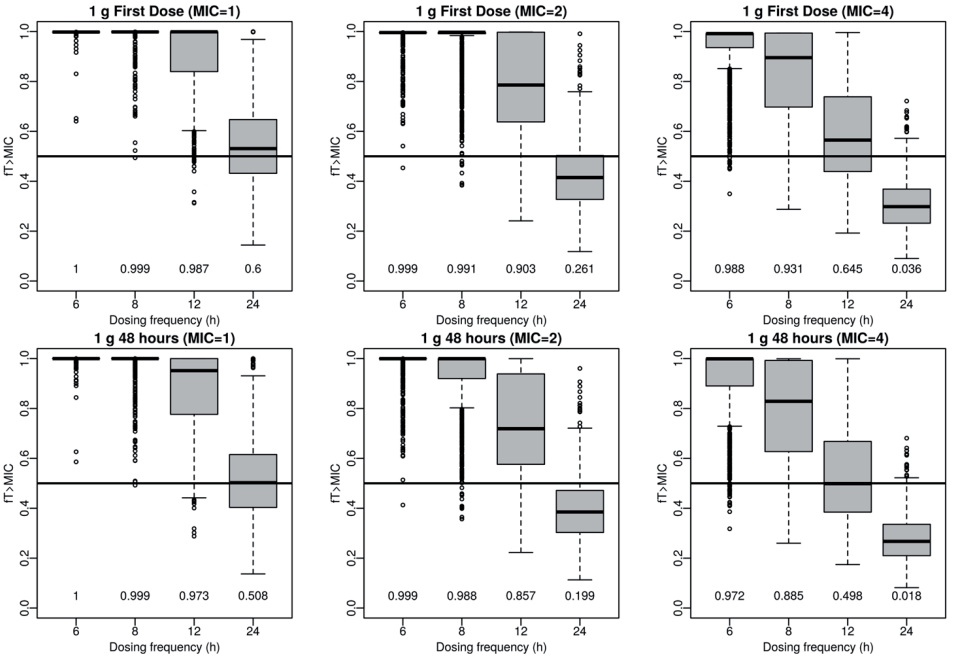

Figure 6. Box and whisker plots for fT>MIC of 1 μg/mL (left), 2 μg/mL (middle) and 4 μg/mL (right) for different dosing frequencies of 1 g of doripenem after 1 dose and after 48 hours. These figures assume MIC doubles over the first 48 hours of treatment. Probability of target attainment given at the bottom of each plot with the target set at fT>MIC of 50%. The bottom and top of the box represent the first and third quartile, respectively, the line inside the box represents median, the whiskers represent the box quartile +/– the interquartile range and the open circles represent outliers.

5.5 Substudy II. Pharmacokinetics of piperacillin/tazobactam during high volume haemodiafiltration

5.5.1 Non-compartmental PK analysis

Ten subjects were included in the PK analysis. The plasma concentration time profiles of piperacillin and especially tazobactam demonstrated high inter-individual variability (Figure 7). No increase of serum concentrations after the end of HVHDF (19 samples from 5 patients) was seen (Figure 7).

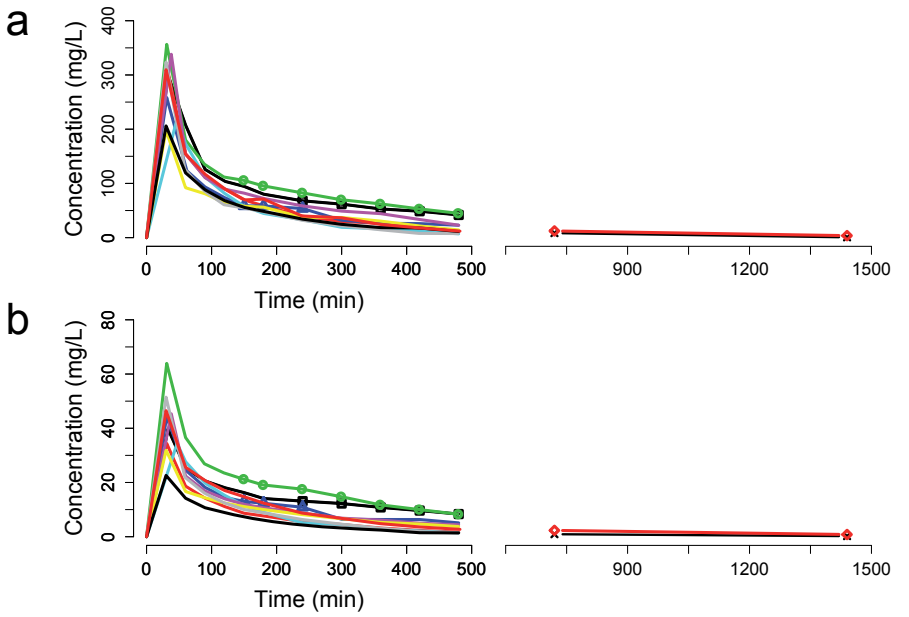

Figure 7. Plasma concentration – time profiles of (a) piperacillin and (b) tazobactam. The open circles, squares and triangles mark measured concentrations off HVHDF

The PK parameters of piperacillin and tazobactam in the study patients are shown in Tables 8 and 9.

Table 8. Noncompartmental PK parameters of piperacillin during HVHDF.

Patient	C_{max} (mg/L)	K_{el} (/h)	$T_{1/2}$ (h)	V_d (L)	AUC_τ (mg*h/L)	AUC_{inf} (mg*h/L)	CL (L/h)
ID-1	299.7	0.291	2.4	19.1	638.9	703.3	6.3
ID-2	317.0	0.328	2.1	19.6	525.0	561.6	7.6
ID-3	356.4	0.484	1.4	13.5	571.4	585.0	7.0
ID-4	258.4	0.241	2.9	32.3	482.8	577.6	8.3
ID-5	219.9	0.365	1.9	23.0	411.2	430.4	9.7
ID-6	338.2	0.328	2.1	21.2	621.8	691.9	6.4
ID-7	197.8	0.384	1.8	30.7	417.3	454.1	9.6
ID-8	323.7	0.386	1.8	19.7	428.3	447.9	9.3
ID-9	206.1	0.287	2.4	29.3	402.0	441.3	9.9
ID-10	309.7	0.361	1.9	18.7	537.2	570.8	7.4
Mean (SD)	282.8 (57.7)	0.346 (0.064)	2.1 (0.4)	22.7 (6.1)	503.6 (88.7)	546.4 (101.0)	8.2 (1.4)

AUC_{inf} – area under the time-concentration curve extrapolated to infinity; AUC_τ – area under the time-concentration curve during the dosing interval; CL – total body clearance; C_{max} – maximal concentration; K_{el} – elimination rate constant; $T_{1/2}$ – terminal elimination half life ; V_d – volume of distribution

Table 9. Noncompartmental PK parameters of tazobactam during HVHDF.

Patient	C_{max} (mg/L)	K_{el} (/h)	$T_{1/2}$ (h)	V_d (L)	AUC_τ (mg*h/L)	AUC_{inf} (mg*h/L)	CL (L/h)
ID-1	39.7	0.248	2.8	18.5	62.6	119.3	4.9
ID-2	34.4	0.333	2.1	21.3	64.9	69.8	7.7
ID-3	63.9	0.447	1.6	9.3	68.0	120.6	4.3
ID-4	44.1	0.212	3.3	24.0	94.7	118.7	5.3
ID-5	34.4	0.376	1.8	17.5	72.0	75.7	6.9
ID-6	45.3	0.183	3.8	25.5	89.9	113.9	5.6
ID-7	31.9	0.199	3.5	30.0	76.7	95.5	6.5
ID-8	51.5	0.370	1.9	14.8	77.4	81.1	6.5
ID-9	22.6	0.325	2.1	30.1	48.1	52.4	10.4
ID-10	46.5	0.281	2.5	15.5	97.2	106.9	5.1
Mean (SD)	41.4 (11.6)	0.297 (0.087)	2.5 (0.8)	20.6 (6.8)	75.1 (14.7)	95.4 (24.3)	6.3 (1.8)

AUC_{inf} – area under the time-concentration curve extrapolated to infinity; AUC_τ – area under the time-concentration curve during the dosing interval; CL – total body clearance; C_{max} – maximal concentration; K_{el} – elimination rate constant; $T_{1/2}$ – terminal elimination half life ; V_d – volume of distribution

5.5.2 Population PK analysis

A 2-compartment model best fitted both the piperacillin and tazobactam on HVHDF concentration–time data (101 samples from 10 patients). Goodness of fit plots and visual predictive check of the final model are shown in Figures 8 and 9. Inclusion of the tested covariates (serum creatinine, APACHE II, effluent rate, effluent volume and fluid removal volume) did not significantly improve the model fit and they were not retained in the final model.

The PK parameter estimates of the final piperacillin and tazobactam PK models are shown in Table 10.

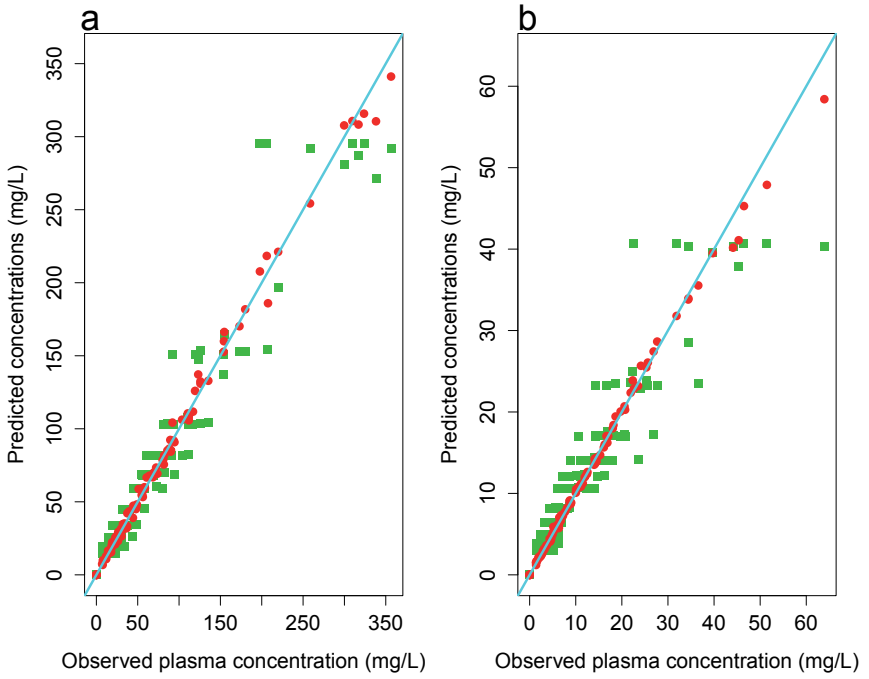

Figure 8. Goodness of fit plots from the final (a) piperacillin and (b) tazobactam population PK model. Red hexagons show individual predictions, green squares show population predictions

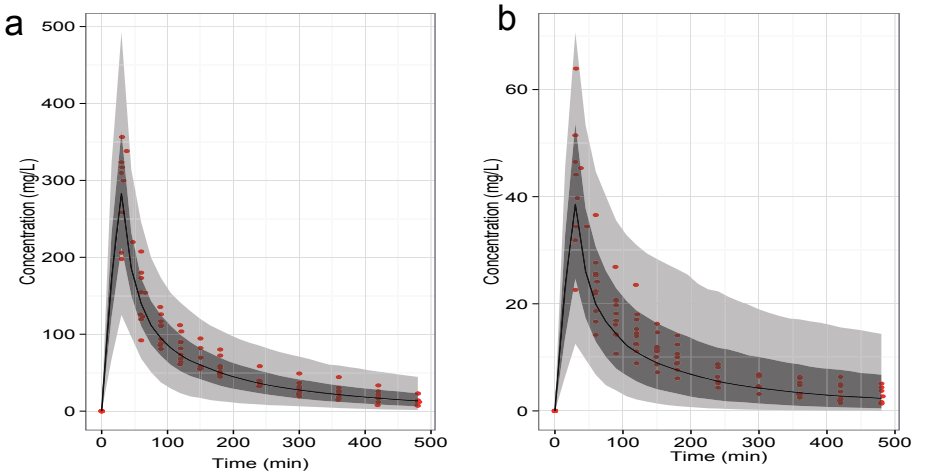

Figure 9. Visual predictive check of the final (a) piperacillin and (b) tazobactam population model. The solid middle line marks the median, the dark grey shaded area marks the interquartile range and the light grey shaded area marks the 95% confidence interval of model predicted data. The red circles represent the observed data

Table 10. The PK parameter estimates of the final PK model

	Piperacillin			Tazobactam		
Parameter	**Estimate**	**95% CI**		**Estimate**	**95% CI**	
		Lower	**Upper**		**Lower**	**Upper**
Fixed effects, population mean PK parameters						
CL L/h (%SE)	6.9 (6.4)	6.1	7.9	5.1 (11.0)	4.1	6.3
V_1 L (%SE)	9.0 (10.1)	7.4	11.0	8.6 (11.0)	6.9	10.7
V_2 L (%SE)	11.2 (12.2)	8.9	14.2	8.9 (15.2)	6.6	12.0
Q L/h (%SE)	10.5 (14.4)	7.9	14.0	9.7 (19.5)	6.6	14.2
Random effects, interindividual variability						
ηC_L	0.19	0.11	0.33	0.33	0.20	0.55
ηV1	0.26	0.14	0.48	0.31	0.18	0.53
ηV2	0.34	0.19	0.60	0.45	0.27	0.75
ηQ	0.37	0.20	0.70	0.57	0.34	0.93
Residual variability, constant plus power of the variance covariate model				**Residual variability, exponential variance function**		
Constant (fixed)	1	–	–	–	–	–
Residual (SD)	0.06	0.03	0.12	0.17	0.14	0.20
Power parameter	1.07	0.86	1.29	–	–	–
Exponential (fixed)	–	–	–	0.07	–	–

CL – total body clearance; Q – intercompartmental clearance; V_1 – volume of distribution of the central compartment; V_2 – volume of distribution of the peripheral compartment; η – fitted value covariate; %SE – percent of standard error

5.5.3 Probability of target attainment

Figure 10 demonstrates the spread of the fT>MIC for each simulated patient for the tested dosing regimens. The probability of the 100%T>MIC target attainment for piperacillin/tazobactam 4.5 g dosed every 6 and 8 hours as 4-hour infusion were 88.6%, and 61.0% respectively for MIC 16 mg/L.

The median, 10th and 90th percentiles of simulated concentration profiles of tazobactam are shown in Figure 11.

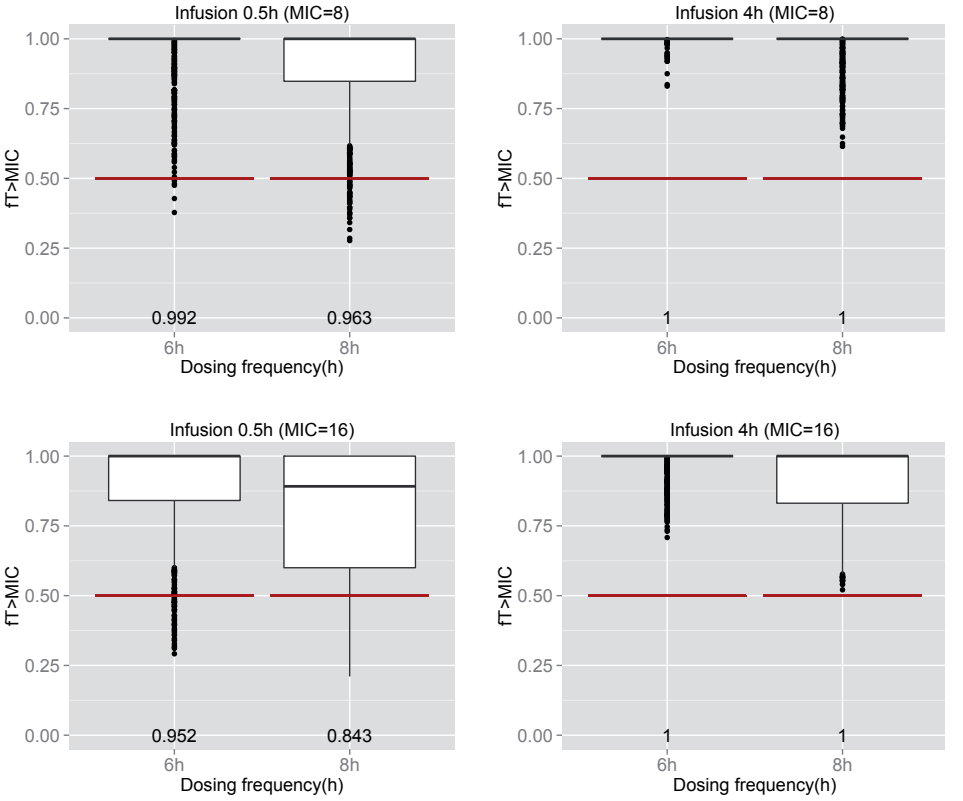

Figure 10. Box and whisker plots for fT>MIC of 8 mg/L (upper) and 16 mg/L (lower) for different dosing frequencies and infusion durations of 4.5 g of piperacillin/tazobactam. The bottom and top of the box represent first and third quartile, respectively, the line inside the box represents median, the whiskers represent the box quartile +/– the interquartile range and the black circles represent outliers. Probability of target attainment given at the bottom of each plot with the target set at fT>MIC of 50%

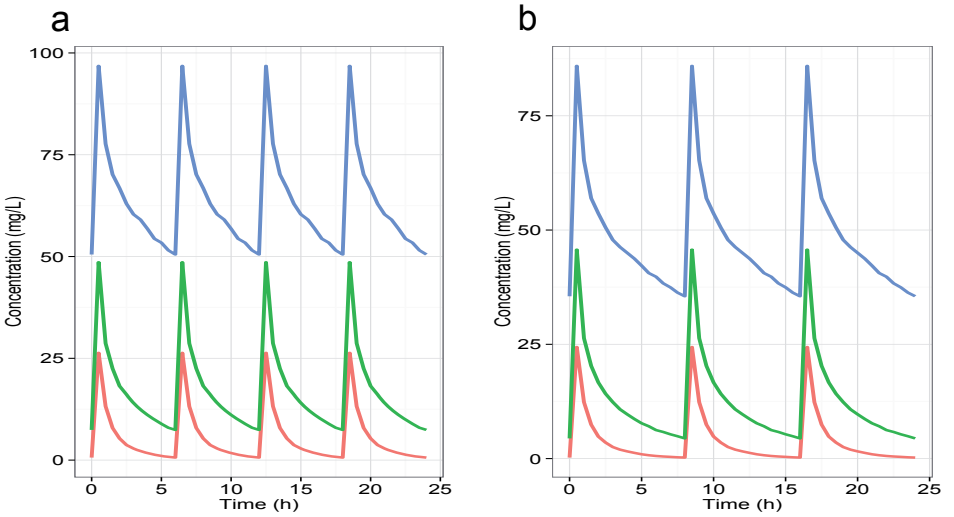

Figure 11. Simulated steady state concentrations of tazobactam 0.5 g administered (a) every 6 hours and (b) every 8 hours. The green line represents median, the red line represents the 10th and the blue line represents the 90th percentile

5.6 Substudy III. Modification of inflammatory response by high volume haemodiafiltration in patients with severe sepsis and septic shock

5.6.1 Metabolic and haemodynamic indices

The changes in haemodynamic and metabolic parameters are shown in Table 11. The dose of norepinephrine required to maintain MAP within the target range of 70–80 mmHg decreased from 0.40 (0.43) μg/kg/min to 0.28 (0.33) μg/kg/min during HVHDF (p=0.009). There were no statistically significant changes in heart rate, MAP, CI, body temperature or serum lactate concentrations. Serum pH increased from 7.36 (0.07) to 7.4 (0.06), p= 0.013.

Table 11. Effects of HVHDF on haemodynamic and clinical variables.

	Pre-HVHDF	Post-HVHDF	p
Mean arterial pressure(mmHg)	89 [71–91]	80 [73–93]	NS
Cardiac index (L/min/m^2)	3.02 [2.5–4.2]	2.9 [2.5–3.3]	NS
Heart rate (beats/min)	102.4 (26.8)	98.5 (22.9)	NS
Body temperature (°C)	37.1 (0.6)	36.9 (0.6)	NS
Arterial pH	7.36 (0.07)	7.40 (0.06)	0.013
Noradrenaline dose (μg/kg/min)	0.40 (0.43)	0.28 (0.33)	0.009
Lactate (mmol/L)	1.5 [1.2–3.0]	1.5 [1.2–2.1]	NS
SOFA	10 [7–11]	9 [7–12]	NS

SOFA – sequential organ failure score. Data presented as mean (SD) or median [interquartile range]

5.6.2 Cytokines

Individual changes in main proinflammatory cytokines are shown in Figure 12, and anti-inflammatory cytokines in Figure 13. While high individual variability was noted, no significant differences before and after HVHDF were observed in the measured cytokines (Table 12). The mean values of most pro- and anti-inflammatory cytokines were substantially higher than those of healthy volunteers (Karu et al. 2013), indicating the presence of systemic inflammatory response in our patients (Table 13). The ratios of pro- and anti-inflammatory mediators (IL-10/IL-6 and IL-10/TNFα) were not influenced by HVHDF.

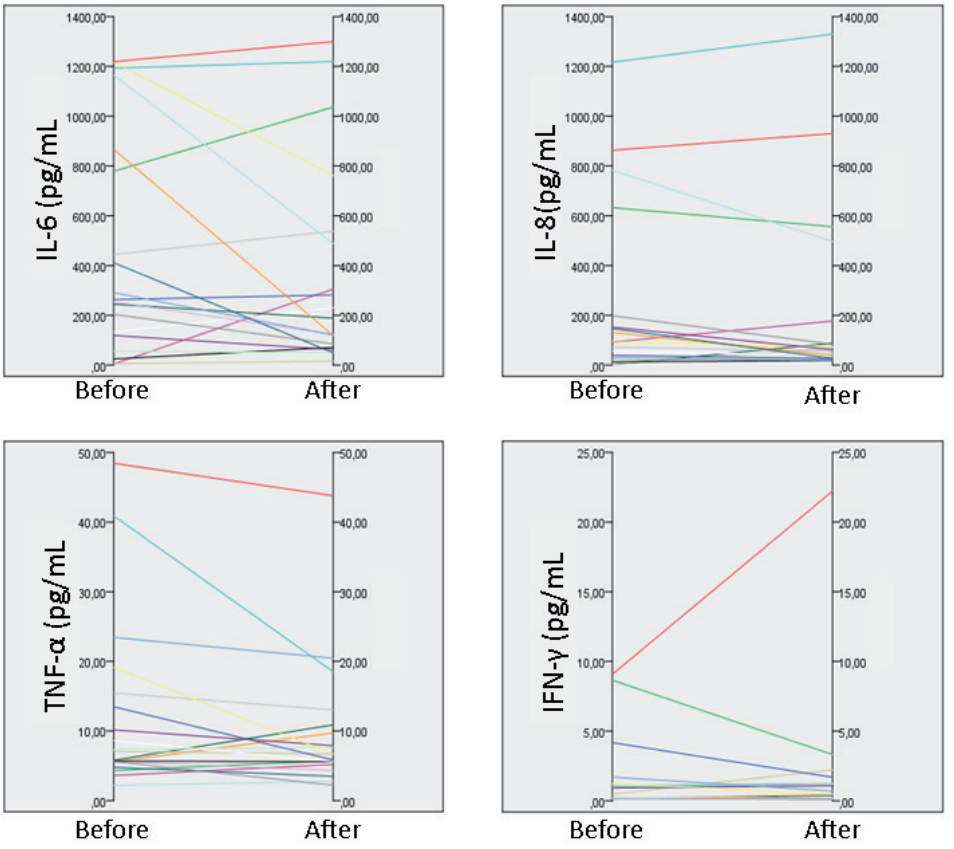

Figure 12. Values of main proinflammatory cytokines before and after high volume haemodiafiltration (HVHDF). The lines represent single patient values.

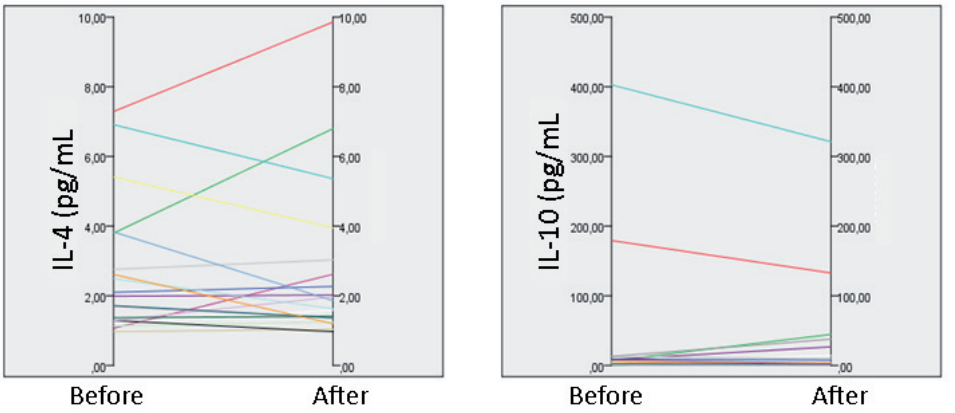

Figure 13. Values of main anti-inflammatory cytokines before and after high volume haemodiafiltration (HVHDF). The lines represent single patient values.

Table 12. Cytokine and growth factor concentrations before and after HVHDF.

Marker	Pre-HVHDF	Post-HVHDF	p
IL-1α	0.2 (0.15–0.31)	0.15 (0.11–0.24)	NS
IL-1β	1.69 (1.35–2.85)	2.34 (1.19–3.27)	NS
IL-2	1.69 (1.55–1.95)	1.76 (1.41–2.05)	NS
IL-4	1.99 (1.29–3.8)	1.86 (1.36–3.03)	NS
IL-6	263.06 (119.11–866.97)	188.44 (71.04–537.83)	NS
IL-8	92.43 (19.16–198.35)	62.25 (30.92–198.35)	NS
IL-10	6.9 (1.81–9.24)	4.22 (2,74–26.8)	NS
TNF-α	7.04 (5.56–15.46)	6.69 (5.2–10.88)	NS
IFN-γ	0.23 (0.14–1.26)	0.25 (0.16–1,26)	NS
VEGF	27.41 (13.66–52.01)	27.23 (13.26–32.48)	NS
EGF	0.87 (0.82–1.04)	0.9 (0.79–1.13)	NS
MCP-1	525.08 (205.95–1090.9)	526.84 (306.12–983.77)	NS

Table 13. Concentrations of cytokines in patients with severe sepsis and septic shock and in healthy volunteers (Karu et al. 2013). Values are presented as median (interquartile range).

	Study patients (n=19)	Healthy volunteers (n=39)*	p
IL-1α (pg/mL)	0.20 (0.15–0.31)	0.10 (0.07–0.21)	0.001
IL-1β (pg/mL)	1.69 (1.35–2.85)	0.66 (0.47–0.93)	0.001
IL-2 (pg/mL)	1.69 (1.55–1.95)	1.67 (1.10–2.36)	0.004
IL-4 (pg/mL)	1.99 (1.29–3.8)	1.38 (1.09–1.78)	0.093
IL-6 (pg/mL)	263.06 (119.11–866.97)	0.73 (0.57–1.00)	<0.001
IL-8 (pg/mL)	92.43 (19.16–198.35)	6.63 (5.13–8.04)	<0.001
IL-10 (pg/mL)	6.9 (1.81–9.24)	0.64 (0.51–0.80)	<0.001
TNFα (pg/mL)	7.04 (5.56–15.46)	2.89 (2.49–3.86)	<0.001
IFNγ (pg/mL)	0.23 (0.14–1.26)	1.37 (1.24–1.74)	0.117

* Data from I. Karu

5.6.3 Sublingual microcirculation

The videos of sublingual microcirculation were recorded in ten patients and were analysable in nine. In one patient the quality was poor because of pressure artefacts and over lighting. No significant effects were observed on measured microcirculatory parameters (Table 14).

Table 14. Sublingual microcirculatory parameters before and after HVHDF.

Parameter	Before HVHDF	After HVHDF	p
Total vascular density	19.4 (16.5–24.8)	21.8 (18.1–26.3)	0.139
Perfused vessel density	18.6 (15.9–22.5)	19.8 (17.7–22.9)	0.173
Proportion of perfused vessels	93.0 (88.8–95.7)	94.4 (90.3–97.1)	0.515
Microvascular flow index	2.8 (2.7–3.0)	2.9 (2.8–3.0)	0.074
DeBacker score	11.4 (9.9–14.7)	13.2 (11.1–15.2)	0.214

Data presented as median and interquartile range.

6. GENERAL DISCUSSION

6.1 Pharmacokinetics of β-lactam antibiotics during high volume haemodiafiltration in patients with septic shock

We demonstrated that in patients with severe sepsis and septic shock with acute kidney injury the total body clearance of both doripenem and piperacillin during HVHDF was about twice slower than in healthy volunteers with normal renal function: doripenem clearance 6.8 L/h vs. 14.6 L/h (Cirillo et al. 2009) and piperacillin clearance 6.9 L/h vs 15.3 L/h (Tjandramaga et al. 1978). Whether the results of the present study can be extrapolated to the recommendations for other carbapenems during HVHDF, remains speculative. The chemical characteristics and PK of meropenem are very close to doripenem (similar terminal elimination half-life, low protein binding, predominantly renal elimination and metabolism via hydrolysis of the beta-lactam ring to a pharmacologically inactive metabolite) therefore, it is rational to expect their similar behaviour also in the setting of HVHDF.

This finding is in contrast with common, yet untested belief that applying very high filtration volumes results in very high antibiotic clearance. Both antibiotics are significantly eliminated by RRT and while the elimination rate depends on the dose of haemo(dia)filtration (Valtonen et al. 2001, Ohchi et al. 2011, Jamal et al. 2014b, Bauer et al. 2012), other RRT settings like blood flow rate, and pre- or postfilter substitution have influence as well. While compared to patients with severe renal impairment undergoing RRT in conventional doses of 1–2 L/h in predilution mode, total body clearance of the antibiotics in our study patients was much higher (Roberts et al. 2014d, Bauer et al. 2012, Asin-Prieto et al. 2014). The difference was smaller, when compared to the setting where substitution fluid was administered post filter (Cirillo et al. 2011, Varghese et al. 2014). Prefilter dilution of the blood results in decreased substance clearance (Ronco et al. 2000), which might explain why the clearance of the both studied antibiotics in our study was only slightly higher than in patients undergoing CVVHDF with much smaller filtration volumes. Yet, even in similar RRT settings with postfilter substitution, extracorporeal clearance of β-lactams has been found highly variable with decreasing effluent-to-plasma concentration ratio with increasing effluent rate (Roberts DM et al. 2015).

Of note, the PK of hydrophilic antibiotics is much influenced by patient factors like residual renal function and severity of illness (Jamal et al. 2015b). Roberts and colleagues found that while proportion of systemic clearance due to RRT varied widely between patients and occasions, the dose of haemodiafiltration did not influence the total body clearance or volume of distribution of β-lactam antibiotics (Roberts DM et al. 2015). One of the reasons for this might be variable residual renal function of the patients. It has been described that

with better renal clearance of creatinine the proportion of extracorporeal clearance of β-lactam antibiotics decreases, and the sum effect is increased total body clearance of the drug (Arzuaga et al. 2005, Asin-Prieto et al. 2014). Other possible explanation of such observation include physiological changes typical to critical illness, like interstitial fluid accumulation, hypoalbuminaemia, disturbed (micro)circulation and liver function (Roberts DM et al. 2012).

6.2 Pharmacokinetics and pharmacodynamics of tazobactam

Sufficient levels of beta-lactamase inhibitor are needed to ensure treatment efficacy and even more importantly to avoid amplification of resistant mutants over time (Lister et al. 1997, Louie et al. 2012). In our study the total body clearance of tazobactam was slower compared to piperacillin. Simulated median tazobactam concentrations were well above 4 mg/L, the concentration used for in vitro piperacillin/tazobactam susceptibility testing (EUCAST 2014). Modelled time-concentration profiles suggest very high steady state levels in some patients. While some studies show accumulation of tazobactam on RRT (Mueller et al 2002, Asin-Prieto et al. 2014), this was not the case during HVHDF. However, high steady state levels, seen in some patients, rise concern about potential toxicity. Tazobactam has been shown to be well tolerated in animal experiments with no observed adverse effect level in dogs as high as 40 mg/kg/day (European Chemical Agency 2014). No data about safe concentrations in humans are available, but no toxic effects have been described either. Avoiding high peak tazobactam concentrations would be an argument to prefer prolonged infusions. Decreasing tazobactam dose in relation to that of piperacillin might be safer yet may lead to tazobactam underdosing and decreased antibacterial activity of the combination (Mueller et al. 2002, Asin-Prieto et al. 2013).

6.3 Are there clear benefits of prolonged or continuous infusion

Numerous studies have shown better PK/PD target achievement with prolonged or continuous infusion of the studied antibiotics (Bhavnani et al 2005; Samtani et al. 2012, Asin-Prieto et al. 2014, Roberts et al 2010a, Felton et al. 2012, Jamal et al. 2015c).Yet, no clinical benefit from the continuous infusion of meropenem, piperacillin/tazobactam and ticarcillin/calulanic acid, compared to intermittent administration was found in a recently published randomised controlled trial (Dulhunty et al. 2015). For piperacillin/tazobactam, evidence from smaller or retrospective studies (Dulhunty et al 2013b, Lodise et al. 2007) and a meta-analysis of mostly nonrandomized trials (Falagas et al. 2013)

suggest clinical benefit; therefore we modelled piperacillin/tazobactam prolonged infusion. In line with other studies (Asin-Prieto et al. 2014, Roberts et al 2010a, Felton et al. 2012, Jamal et al. IJAA 2015c) we found that this method of delivery ensures better PK/PD target attainment. For doripenem, the real clinical benefit from continuous infusions is even less probable. On the contrary, a RCT of ventilator-associated pneumonia with higher death rates in doripenem 4 hour infusion group compared with 1 hour infusion of imipenem has been published (Kollef et al. 2012). The authors state that the imipenem arm had less mortality because it was a 10-day course as opposed to 7-day course of doripenem, but we cannot rule out the possibility that low maximal concentration with long infusion leads to low tissue penetration and therefore even though circulating concentrations are above MIC, we do not have sufficient concentrations in the tissues. Therefore we did not model pharmacodynamic target attainment with doripenem.

Starting antibacterial therapy with prolonged or continuous infusion may delay achieving of therapeutic drug concentrations (Rhodes et al. 2014, De Waele et al. 2015). This shortcoming can be overcome by providing a bolus loading dose, followed by prolonged infusion (De Waele et al. 2015). Some studies comparing continuous and intermittent administration of antibiotics have used loading doses (Dulhunty et al. 2013a, Roberts et al. 2009a, Dulhunty et al. 2015), while others have not (Roberts et al. 2009b). Loading doses should be used with prolonged antibiotic administration to ensure quick achievement of target plasma concentration and provide higher concentration gradient that enables more rapid achievement of therapeutic concentration at the site of infection (Rhodes et al. 2014, Roberts et al. 2014b) Other problems with prolonged infusion of antibiotics include drug stability, dead space of administering devices and drug to drug incompatibility (DeWaele et al. 2015). While meropenem is stable in 0.9% NaCl for 8 hours at temperatures less than 25°C (Carlier et al. 2015), in glucose solutions carbapenems cannot be infused for longer than three hours (DeWaele et al. 2015). The problem of excessive dead space can be overcome by using syringe pumps, while guaranteeing a dedicated line for antibiotic infusion to avoid incompatibilities, may sometimes be difficult (De Waele et al. 2015).

In summary, while the PK/PD targets are better met with prolonged carbapenem and piperacillin/tazobactam infusions, no clinical benefit has been shown.

6.4 Dosing of doripenem and piperacillin/tazobactam during high volume haemodiafiltration

For PK/PD target we chose 50%T>MIC, which for β-lactam antibiotics has been associated with clinical cure in intensive care patients (Roberts et al. 2014c). For both antibiotics, doses recommended by the manufacturer (EMA,

Doribax; EPAR; Pfizer Ltd. 2013) for adult patients with normal renal function, ensured > 50%T>MIC for susceptible bacteria and could be recommended during extended daily HVHDF. As both antibiotics are predominantly used to treat infections caused by more resistant bacteria like *Pseudomonas* or ESBL-producing enterobacteria (Harris et al. 2015), and severe sepsis can induce immunosuppression (Angus et al. 2013), higher doses may be needed to achieve clinical cure and avoid resistance development.

Similar to other studies of critically ill patients (Bauer et al. 2012, Roberts et al. JAC 2014, Asin-Prieto et al. 2014, Roberts DM et al. 2015) we found high inter-patient variability in doripenem, piperacillin and tazobactam PK parameters, which may lead to underdosing or toxicity in some patients. Individualised dosing for these patients has been recommended (Roberts et al. 2014a, Jamal et al. 2015b). For individualised dose calculations mathematical modelling is one possibility. Yet modelling is extremely complicated due to huge number of patient and RRT variables with varying magnitude of influence involved (Jamal 2015b, Roberts DM 2015). At present, therapeutic drug monitoring (TDM) seems to be the most promising method for ensuring that antibiotic therapeutic targets are achieved in critically ill patients receiving RRT (Roberts DM 2015).

6.5 Tissue perfusion during high volume haemodiafiltration

Since the landmark study by Ronco and colleagues showing better survival of critically ill patients with higher filtration volumes (Ronco et al. 2000), high volume haemofiltration has been used in patients with septic shock in order to achieve haemodynamic stabilisation and restore homeostasis. Pulse high volume haemofiltration has been proposed as salvage therapy for patients in severe septic shock, whose haemodynamics cannot be stabilised with conventional therapy (Honore et al. 2000, Cornejo et al. 2006). We found significant decrease of norepinephrine dose requirement during extended high volume haemodiafiltration in patients with septic shock despite negative fluid balance in most patients. This finding is in line with other experimental (Bellomo et al. 2000, Yekebas et al. 2002, Sykora et al. 2009) and clinical studies (Honore et al. 2000, Cole et al. 2001, Joannes-Boyau et al. 2004, Cornejo et al. 2006, Boussekey et al. 2008, Chu et al. 2013). However, a recent randomized multicentre study, comparing continuous haemofiltration in the dose of 35 ml/kg/h to 70 mL/kg/h (Joannes-Boyau 2013), did not show any haemodynamic or survival benefit from higher filtration volumes, yet the study was underpowered, recruiting 140 patients instead of 460 required from power calculation.

While the increase on blood pressure during HVHF occurs due to increased vascular resistance rather than increased cardiac output (Cornejo et al. 2006), disturbances of microcirculation might be aggravated (Sykora et al. 2009). Yet,

in line with other studies (Ruiz et al. 2010) we found no negative influence of HVHDF on microcirculation despite fluid removal during the procedure. The patients were volume resuscitated before the study inclusion and had micro-circulation indices close to normal (Maddison et al. 2014).

6.6 Removal of cytokines by high volume haemodiafiltration

As cytokines are water soluble molecules with molecular mass of 5 kDa to 60 kDa, circulating freely in plasma, a wide range of pro and anti-inflammatory cytokines with molecular mass below the membrane cut-off can be removed by ultrafiltration (Rimmele et al. 2012). Yet, significant amounts of cytokines in ultrafiltrate and/or significant decrease in plasma cytokine concentration have been shown in few studies (Sanchez-Izquierdo et al. 1997, Cole et al. 2001, Ghani et al. 2006, Peng et al. 2010, Chu et al. 2013). A meta-analysis of animal studies concluded that high volume haemofiltration with conventional haemofilters effectively removes IL-6 and IL-10, while TNF-α is not removed due to molecular mass above membrane cut-off values (Atan 2013a).

For the polyarylethersulfone (PAES) haemofilter, used in this study, removal of small molecular-weight proteins with molecular mass up to 24 kDa via both filtration and adsorption has been shown in ESRD patients (Ouseph et al. 2008). No data could be found for the use of Polyflux 210H in septic shock patients.

As the efficacy of cytokine removal by HVHF is questionable other mechanisms potentially responsible for the decrease in norepinephrine requirements, induced by HVHF are cooling, which was not the case in our study, removal of unmeasured organic anions (Bellomo et al. 2013) or removal of other mediators, responsible for hypotension and vasodilation, like prostaglandins, leukotrienes (Yokoyama et al. 2009), complement factors (Cole et al. 2001) or myocardial depressants (Blake et al. 1996). Increase in pH might also have been responsible for the decrease of vasopressor requirement. Yet this explanation is unlikely, as Bellomo and colleagues comparing different intensities of continuous haemofiltration (CHF) found similar correction of acidosis, but greater increase in mean arterial pressure and decrease in norepinephrine requirements in high intensity CHF group (Bellomo et al. 2013).

6.7 Methodological considerations

We performed a single dose pharmacokinetic study with rich precisely timed and documented sampling schedule that allows accurate description of plasma concentration-time profiles and efficient population PK parameter estimation of doripenem, piperacillin and tazobactam during HVHDF. It has been shown that

sampling schedule has influence on the efficiency of population PK parameter estimation with inaccurate estimates with low number of data points per individual (al Banna et al. 1990). Yet we did not measure steady state concentrations of the antibiotics. In one study the total body clearance and volume of distribution of piperacillin has been shown to be lower after multiple doses than after a single dose (Capellier et al. 1998). Other studies have not reached similar results (Roberts et al. 2009a, Jamal et al. 2015c). Still, large intra-individual day-to-day variability in total body clearance and RRT clearance of β-lactam antibiotics has been shown, making empirical dosing of these drugs extremely complicated and suggesting the need of therapeutic drug monitoring (Roberts DM et al. 2015). Dynamic changes in physiology of critical illness, especially in residual renal function may contribute to these observations (Roberts DM et al. 2015).

We were not able to describe the extent of extracorporeal clearance of the studies antibiotics as it was not possible to get ultrafiltrate/dialysate samples from the tubing. Still, our data on total exposition to the drugs and total body clearance allow dose recommendations in this population.

A two-compartment linear model fitted both the doripenem and piperacillin/ tazobactam data best. Previous data suggest that piperacillin elimination may have a significant saturable component in healthy volunteers, mostly due to saturable renal tubular secretion (Tjandramaga et al. 1978, Bergan et al. 1982, Landersdorfer et al. 2012). Yet the influence of saturable renal elimination for clinically relevant dosage regimens is thought to be insignificant in healthy volunteers (Landersdorfer et al. 2012) and in critically ill patients (Roberts et al. 2009a). Other studies of the PK of piperacillin in critically ill patients undergoing RRT have also found two-compartment linear model best fitted to the data (Giron et al. 1981, van der Werf et al. 1997, Asin-Prieto et al. 2013).

We were not able to draw conclusions about the impact of patient or HVHDF characteristics on the pharmacokinetics of doripenem, piperacillin and tazobactam. This was beyond the scope of the study. As our aim was to define dosing recommendations, we used a well standardised HVHDF protocol. Also, during haemodiafiltration both diffusive and convective clearances play important role in the elimination of small molecules like the studied antibiotics. Both clearances may be non-linear at higher effluent rates (Roberts DM et al. 2015) and varying proportions of dialysate and ultrafiltrate flows may influence solute clearance variably (Brunet et al. 1999).

Future research could be directed to using therapeutic drug monitoring to identify covariates that influence the PK of antibiotics in critically ill patients, in order to enable model-based individual dosing recommendations, and further, assessing the influence of the individual dosing recommendations on clinical efficacy, safety and possibly on resistance development.

7 CONCLUSIONS

1. Application of extended HVHDF for treatment of acute kidney injury results in considerable removal of doripenem in septic shock patients. Doses of 500 mg every 8 h are necessary for treatment of susceptible bacteria in immunocompetent patients during daily HVHDF, if the sessions are extended to meet the standards of continuous renal replacement therapy. Aiming for more than 50% of time above MIC and/or treating infections with less susceptible micro-organisms, dosing regimens up to 1000 mg every 8 h could be advocated for optimal efficacy during HVHDF.
2. For bactericidal PK/PD target attainment piperacillin/tazobactam doses of 4/0.5 g every 8 hours appear appropriate in severe sepsis and septic shock patients with minimal residual renal function during HVHDF. This dosing regimen is sufficient for treating infections caused by intermediately susceptible bacteria. Further evaluation of the potential toxicity of tazobactam is warranted.
3. Due to high inter-individual variability and insufficient data available for sound individual dose modelling in patients with severe sepsis and septic shock on HVHDF, dose adjustment based on therapeutic drug monitoring of the β-lactam concentrations may be the preferred way to improve therapeutic target attainment.
4. The single-centre study suggests that extended intermittent HVHDF results in decrease of norepinephrine requirement and preserved microcirculation in patients with severe sepsis and septic shock. Haemodynamic improvement is not associated with decrease in circulating cytokine levels. Further research focusing on the identification of the mechanisms underlying these findings may help to potentiate these effects in order to improve treatment outcomes.

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9. SUMMARY IN ESTONIAN

Suuremahuline hemodiafiltratsioon raske sepsise ravis – toime antibiootikumide farmakokineetikale ning süsteemsele põletikureaktsioonile

Sepsis – organismi ülepiiriline reaktsioon infektsioonile – ja selle kõige raskemad vormid, raske sepsis (infektsioonist tingitud äge(dad) organpuudulikkus(sed)) ja septiline šokk (raskele sepsisele lisandunud hüpotensioon, mis ei möödu infusioonraviga) (Dellinger *et al.* 2013), on oluline tervishoiuprobleem (Angus et al. 2013). Raske sepsise esinemissagedus suureneb (Brun Buisson *et al*. 2004). Kuigi surevus on viimase kümnendi jooksul vähenenud, jääb see siiski kõrgeks (Brun Buisson *et al*. 2004, Dombrovskiy *et al.* 2007). Äge neerupuudulikkus, mis sageli raske sepsise tüsistusena tekib, on iseseisev surma riskifaktor (Brun Buisson *et al.* 2004).

Sepsise ravi võtmeküsimusteks on adekvaatne antibakteriaalne ravi, kiire kolde kontroll ja ülepiirilise immunoloogilise reaktsiooni pidurdamine. Antibakteriaalse ravi hiline algus või vale antibiootikumi valik viivad sepsisehaigete surevuse suurenemisele (Kumar et al. 2006). Süsteemse põletikureaktsiooni pidurdamine on niisama oluline, kuid raske saavutada selle reaktsiooni väga keeruka olemuse ja osalevate mediaatorite suure arvu tõttu. Viimasel 15 aastal on immuunhomöostaasi taastamiseks kasutatud mediaatorite kehavälist eemaldamist neeruasendusravi abil (Ronco et al. 2004). Keskmiste molekulide, nagu tsütokiinid eemaldamise efektiivsus sõltub rakendatud neeruasendusravi meetodist, aga ka filtri omadustest ja ravi mahust (Ricci et al. 2006a, Cerda et al. 2009). Kliinilised ja eksperimentaalsed andmed näitavad ägeda neerupuudulikkusega sepsisehaigete paremat elulemust neeruasendusravi mahtude suurenemisega (Honore et al. 2000, Schiffl et al. 2002, Cornejo et al. 2006, Bouman et al. 2007). Andmed on siiski mõneti vastuolulised, sest hiljutine randomiseeritud kontrollitud uuring sellisele tulemusele ei jõudnud (Joannes-Boyau et al. 2013). Kron-i ja kaastöötajate avaldatud esmased tulemused lubavad oletada, et suuremahuline hemodiafiltratsioon spetsiifilises *Ultracontrol* režiimis on seotud haiguse raskuse järgi prognoositust suurema elulemusega (Kron et al. 2011). Erinevalt tavalisest rõhu- või mahukontrollitud hemofiltratsioonist tiitrib *ultracontrol* režiim diafiltratsiooni ajal hemofiltri transmembraanset rõhku nii, et saavutatakse optimaalsed tingimused (maksimaalne filtratsioonimaht madalaima võimaliku rõhu juures). Pakendatud asenduslahuste asemel kasutatakse läbi filtrite puhastatud vett, millele lisatakse elektrolüüdid. See muudab meetodi võimalikuks, kulud ei ületa oluliselt tavalise hemodialüüsi kulusid (Kron et al. 2011).

Kuigi potentsiaalselt efektiivne süsteemse põletikureaktsiooni ravis, võib suuremahuline hemodiafiltratsioon organismist eemaldada liigselt antibiootikume, mistõttu nende eluliselt oluliste ravimite kontsentratsioon vereplasmas ja kudedes langeb alla vajaliku, vähendades või elimineerides protseduurist saadava

potentsiaalse kasu (Jamal et al. 2014b). Teisest küljest võib annuste kontrollimatu suurendamine põhjustada kõrvaltoimeid (Roberts DM et al. 2012).

Beeta-laktaamantibiootikumide farmakokineetikat on uuritud erinevate neeruasenduravi meetodite ajal ja on leitud, et hemo(dia)filtratsioon eemaldab neid olulises hulgas, seejuures sõltub eemaldamine neeruasenduravi meetodist ja mahust (Jamal et al. 2014b). Suuremahulise hemodiafiltratsiooni ajal ei ole nende antibiootikumide farmakokineetikat varem uuritud.

Uurimistöö eesmärgid

Uurimistöö üldiseks eesmärgiks oli kirjeldada suuremahulise hemodiafiltratsiooni mõju kahe β-laktaam-antibiootikumi farmakokineetikale ja süsteemsele põletikureaktsioonile raskest sepsisest või septilisest šokist tingitud ägeda neerupuudulikkusega patsientidel.

Konkreetsed eesmärgid:

1. Kirjeldada doripeneemi farmakokineetikat suuremahulise hemodiafiltratsiooni ajal, et määrata optimaalsed annused selle neeruasendusravi meetodi jaoks
2. Kirjeldada piperatsilliini ja tasobaktaami farmakokineetikat suuremahulise hemodiafiltratsiooni ajal, et määrata optimaalsed annused selle neeruasendusravi meetodi jaoks
3. Teha kindlaks, kas suuremahuline hemodiafiltratsioon mõjutab seerumi tsütokiinide profiili ja/või omab efekti raske sepsise ja septilise šoki haigete arteriaalsele vererõhule või keelealusele mikrotsirkulatsioonile

Patsiendid ja metoodika

Käesolev uurimus põhineb SA Tartu Ülikooli Kliinikumi üldintensiivravi osakonnas 01.09.2011 – 25.06.2014 läbi viidud uuringul.

Uuringusse kaasati täiskasvanud patsiendid, kellel oli raske sepsis või septiline šokk (Bone et al. 1992) sellest tingitud ägeda neerupuudulikkusega, kes raviarsti hinnangul vajasid neeruasendusravi suuremahulise hemodiafiltratsiooniga, kellele oli paigaldatud arteri kanüül ja kelle lähedane oli andnud informeeritud nõusoleku patsiendi uuringus osalemiseks. Suuremahuline hemodiafiltratsioon viidi läbi *ultracontrol* režiimis eellahjendusega (Kron et al. 2011). Verevoolu kiirus hoiti 200 mL/min ja dialüsaadi/asenduslahuse voolukiirus 500–650 mL/min. Hemodiafiltratsiooni planeeritud kestvus oli 10 tundi. Doripeneemi esimese doosi farmakokineetika uuringusse kaasatud üheksale patsiendile manustati 500 mg doripeneemi ühetunnise infusioonina ja võeti vereplasma proovid vahetult pärast infusiooni lõppemist ja iga 30 minuti ning hiljem iga tunni järel kaheksa tunni jooksul. Piperatsilliin/tasobaktaami uuringusse kaasatud 10 patsiendile manustati 4g piperatsilliini koos 0,5g taso-

baktaamiga 30-minutilise infusioonina ja vereplasma proovid koguti vahetult infusiooni lõppedes ja edasi iga 30 minuti ning hiljem iga tunni järel kaheksa tunni jooksul. Kahel patsiendil koguti vereproovid ka pärast hemodiafiltratsiooni lõppu 12 ja 24 tundi ravimi manustamisest. Doripeneemi, piperatsilliini ja tasobaktaami kontsentratsioonid määrati kõrgsurve vedelikkromatograafia ja tandem mass-spektomeetria abil. Saadud plasmakontsentratsioonide alusel modelleeriti doripeneemi, piperatsilliini ja tasobaktaami populatsiooni- farmakokineetika parameetrid suuremahulise hemodiafiltratsiooni ajal ning viidi läbi Monte Carlo simulatsioon suuremahulise hemodiafiltratsiooni ajal vajalike doripeneemi ja piperatsilliini annuste leidmiseks. Kõigil mõlemasse uuringusse kaasatud patsientidel (kokku 19) registreeriti arteriaalne vererõhk, vasopressorite annused ja muud kliinilised parameetrid enne ja pärast hemodiafiltratsiooni, samuti koguti vereseerumi proovid tsütokiinide kontsentratsiooni määramiseks. Kümnel patsiendil registreeriti enne ja pärast hemodiafiltratsiooni keelealune mikrotsirkulatsioon.

Peamised tulemused

Doripeneemi maksimaalsed plasmakontsentratsioonid olid väga suure varieeruvusega: 12,4–40,7 mg/L. Doripeneemi tase plasmas ületas tundlike bakterite minimaalset inhibeerivat kontsentratsiooni (MIC) 1 mg/L kogu määramisperioodi ajal kaheksal haigel üheksast. Doripeneemi kogukliirens ägeda neerupuudulikkusega septilises šokis patsientidel oli suuremahulise hemodiafiltratsiooni ajal umbes kaks korda aeglasem kui tervetel vabatahtlikel. Tundlike bakterite poolt põhjustatud infektsioonide raviks nendel patsientidel selle neeruasendusravi ajal sobivad annused 500 mg iga 8 tunni järel. Kui ravitakse mõõdukalt tundlike bakterite poolt põhjustatud infektsioone või on tegemist neutropeenias patsientidega, võib olla vajalik annuste suurendamine.

Piperatsilliini ja tasobaktaami maksimaalsete kontsentratsioonide varieeruvus oli samuti suur: piperatsilliinil 197,8–356,4 mg/L ja tasobaktaamil 22,6–63,9 mg/L. Ka piperatsilliini kogukliirens ägeda neerupuudulikkusega septilises šokis patsientidel oli tervetest vabatahtlikest umbes kaks korda aeglasem. Suuremahulise hemodiafiltratsiooni foonil sobivad sellele patsientide grupile piperatsilliini annused 4g iga 8 tunni järel nii tundlike kui ka mõõdukalt tundlike bakterite poolt põhjustatud infektsioonide raviks. Standardses kombinatsioonis 4g piperatsilliiniga koos manustatav 0,5 grammi tasobaktaami kindlustab vajalikuks ajaperioodiks selle β-laktamaasi inhibiitori vajaliku kontsentratsiooni. Samas ulatusid modelleeritud tasobaktaami kontsentratsioonid mõnel juhul väga kõrgete väärtusteni, mis võib tõsta ohutuse küsimuse.

Keskmise vererõhu 70–80 mmHg säilitamiseks vajalik noradrenaliini annus vähenes suuremahulise hemodiafiltratsiooni ajal oluliselt [0,40 (0,43) μg/kg/min → 0,28 (0,33) μg/kg/min, p=0,009]. Määratud tsütokiinide kontsentratsioonides ega keelealuse mikrotsirkulatsiooni parameetrites olulisi muutusi ei tekkinud.

Järeldused

1. Doripeneemi annused 500 mg iga 8 tunni järel sobivad tundlike bakterite põhjustatud infektsioonide raviks septilises šokis patsientidel igapäevase suuremahulise hemodiafiltratsiooni ajal, kui protseduuri pikendatakse vähemalt 20 tunnini ööpeäevas. Immuunsupressiivsetel patsientidel või mõõdukalt tundlike bakterite poolt põhjustatud infektsioonide ravis võib olla vajalik annuste suurendamine.
2. Piperatsilliin/tasobaktaami annused 4,5g iga 8 tunni järel on sobivad nii tundlike kui ka mõõdukalt tundlike bakterite põhjustatud infektsioonide raviks septilises šokis patsientidel igapäevase suuremahulise hemodiafiltratsiooni ajal. Tasobaktaami võimalikku toksilisust oleks vaja edasistes uuringutes hinnata.
3. Doripeneemi ja piperatsilliin/tasobaktaami annused tuleks võimalusel igale patsiendile määratud plasmakontsentratsioonide alusel individuaalselt kohandada.
4. Suuremahulise hemodiafiltratsiooni rakendamine ägeda neerupuudulikkusega septilises šokis patsientidel vähendas arteriaalse vererõhu säilitamiseks vajalikku noradrenaliini annust. Hemodünaamika paranemine ei olnud seotud tsirkuleerivate tsütokiinide kontsentratsiooni vähenemisega, keelealuses mikrotsirkulatsioonis muutusi ei tekkinud.

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PUBLICATIONS

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Publications relevant to the thesis:

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ELULOOKIRJELDUS

Nimi: Kadri Tamme
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Praegune töökoht, amet: SA TÜ Kliinikum, Anestesioloogia ja intensiivravi kliinik, 1. intensiivravi osakond, arst-õppejõud,
TÜ Anestesioloogia ja intensiivravi kliinik, vanemassistent

Haridus:

1975–1983	M. Härma nim. Tartu II Keskkool
1983–1989	Tartu Ülikool, arstiteaduskond – diplom *cum laude*
1989–1990	Tartu Kliiniline Haigla, anestesioloogia ja intensiivravi internatuur – anestesioloog
1993–1997	Tartu Ülikool, arstiteaduskond, doktoriõpe
2011–2015	Tartu Ülikool, arstiteaduskond, doktoriõpe

Keelteoskus: eesti, inglise, vene

Töökogemus (teenistuskäik):

1990–…	SA TÜ Kliinikum, Anestesioloogia ja Intensiivravi Kliinik, üldintensiivravi osakond, arst-õppejõud
2005–…	Tartu Ülikool, Arstiteaduskond, Anestesioloogia ja intensiivravi kliinik, vanemassistent
2003–2004	Quintiles Estonia OÜ, kliiniliste uuringute koordinaator
1999–2003	Quintiles Estonia OÜ kliiniliste uuringute monitor

Peamised uurimisvaldkonnad:
täiskasvanu sepsis – etioloogia, hulgiorganpuudulikkuse patogeenes: oksüdatiivne stress, mikroorganismide translokatsioon; antibakteriaalse ravi doseerimine; seedetrakti puudulikkus, intraabdominaalne hüpertensioon.
14 publikatsiooni

Doktoritööga seotud publikatsioonid:

Tamme K, Oselin K, Kipper K, Low K, Standing JF, Metsvaht T, Karjagin J, Herodes K, Kern H, Starkopf J. Pharmacokinetics of doripenem during high volume hemodiafiltration in patients with septic shock. J Clin Pharmacol. 2015; 55: 438–446

Tamme K, Oselin K, Kipper K, Tasa T, Metsvaht T, Karjagin J, Herodes K, Kern H, Starkopf J. Pharmacokinetics and pharmacodynamics of piperacillin/tazobactam during high volume haemodiafiltration in patients with septic shock. Acta Anaesthesiol Scand. 2015; doi: 10.1111/aas.12629

Tamme K, Maddison L, Kruusat R, Ehrlich HE, Viirelaid M, Kern H, Starkopf J. Effects of High Volume Haemodiafiltration on Inflammatory Response Profile and Microcirculation in Patients with Septic Shock. BioMed Res Int. 2015, Article ID 125615, http://dx.doi.org/10.1155/2015/125615

Saadud uurimistoetused ja stipendiumid: osalenud põhitäitjana projektides „Gastrointestinaalne puudulikkus ja intra-abdominaalne hüpertensioon intensiivravi haigetel" (ETF6950), "Antibiootikumide doseerimine raskete infektsioonide korral" (3.2.1001.11-0032), ja täitjana projektides "Infektsioon immuunsüsteemi häirega isikutel – haiguse mehhanismidest kliinilise praktikani" (SF0180004s12) ja "Infektsioonid langenud immuunsusega isikutel – eksperimendist kliinilisse praktikasse" (IUT34-24).

Muu teaduslik organisatsiooniline ja erialane tegevus (konverentside ettekanded, osalemine erialastes seltsides, seadusloome jms.): Ettekanded konverentsidel: Stendiettekanded: "Pharmacokinetics (PK) of doripenem during high volume haemodiafiltration in septic shock"; Euroopa Intensiivravi Seltsi aastakongress Pariis 2013; "Pharmacokinetics of piperacillin and tazobactam during high volume haemodiafiltration (HVHDF) in septic shock." ICAAC, Washington 2014. Loengud: "Pharmacokinetics of antibiotics during renal replacement therapy in sepsis" loeng Balti anestesioloogide kongress, Vilnius 2012; "How to dose antibiotics during haemodiafiltration" Balti anestesioogide kongress, Riia 2014.

Eesti Arstide Liidu, Eesti Anestesioloogide Seltsi, Euroopa Intensiivravi Seltsi (ESICM) liige

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
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5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
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