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Falsely Elevated Cyclosporine A and Tacrolimus Concentrations due to Reversible Adsorption to Central Venous Catheters

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1. Abbreviations

C_o	Trough level
C_{max}	Peak level
CNI	Calcineurin inhibitor (here: CsA and Tac)
CsA	Cyclosporine A
CVC	Central venous catheter
CYP3A	Cytochrome P450, family 3, subfamily A
EDTA	Ethylenediaminetetraacetic acid
FFP	Fresh Frozen Plasma
FKBP-12	FK506-binding protein-12
GVHD	Graft-versus-host disease
HPLC	High performance liquid chromatography
I.v.	Intravenous
LC/MS/MS	Liquid chromatography/tandem mass spectrometry
NaCl	Sodium chloride (0.9 %)
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
P0	First 2 mL portion of the mimicked in vitro blood sampling (discarded)
P1 – P5	Consecutive 2 mL portions of the mimicked in vitro blood sampling
PICC	Peripherally inserted central venous catheters
PU	Polyurethane

PU-AH-CVC	Polyurethane “Quad-Lumen Central Venous Catheterization Set”, Arrow-Howes™
PU-In-CVC	Polyurethane “Trilucath” central venous catheter, Intra
PVC	Polyvinyl chloride
Silicone-Vy-CVC	Silicone “Lifecath Apheresis Plus” central venous catheter, Vygon
Silver-Vy-CVC	“Multicath 4 Expert” central venous catheter made of polyurethane incorporated with silver ion-based antimicrobial agent, Vygon
Tac	Tacrolimus
TDM	Therapeutic drug monitoring
TTP/HUS	Thrombotic thrombocytopenic purpura / hemolytic uremic syndrome
ZVK	Zentralvenenkatheter

2. Introduction

2.1. Immunosuppressive Therapy

Transplantation is one of the key components of modern medicine. In 2013, a total reported number of 28,954 solid organ transplantations was performed in the US alone (Transplants by Donor Type). None of these lives could have been saved without reliable immunosuppressive therapy following transplantation. Today, the following classes of immunosuppressive drugs are prescribed to patients following solid organ or allogeneic stem cell transplantation or to those suffering from severe auto-immune diseases: glucocorticoids, calcineurin inhibitors (CNIs), anti-proliferative/antimetabolic agents and biologicals (e.g. antibodies). Typically, a combination of three drugs from different classes is administered simultaneously consisting of a CNI, glucocorticoids and mycophenolic acid, a purine metabolism inhibitor (Geissler and Schlitt 2009; Krensky et al. 2011).

For almost 30 years, the two calcineurin inhibitors cyclosporine A (CsA) and tacrolimus (Tac) have been cornerstones in immunosuppressive therapy after solid organ as well as allogeneic stem cell transplantation (Barbarino et al. 2013; Starzl et al. 1989; Storb et al. 1986). Although they have made a difference in thousands of patients' lives over the years, it is essential to abide by specific rules of these immunosuppressive drugs. In order to ensure their intended effect, the concentration of drugs like CsA and Tac in the patient's blood has to be kept within narrow therapeutic ranges (UpToDate: Cyclosporine (systemic): Drug information 2015; UpToDate: Tacrolimus (systemic): Drug information 2015). If the concentrations are too high, the patients are at risk for nephrotoxicity, hypertension or even life-threatening infections. Low concentrations of CNIs, on the other hand, can result in graft-versus-host disease (GVHD) or transplant rejection (Geissler and Schlitt 2009; Group 1994; Ram et al. 2012). Therefore, it is crucial to maintain the correct blood concentrations of CsA and Tac in order not to put the patient's life at risk.

2.1.1. Cyclosporine A

CsA, a cyclic peptide consisting of eleven amino acids with a molecular weight of 1,202 daltons, was discovered in the 1970s and introduced as a medical drug in the early 1980s (Borel et al. 1976; Choc 1997). It is produced by the fungus *Polypocladium inflatum* (Schild and Förstermann 2013).

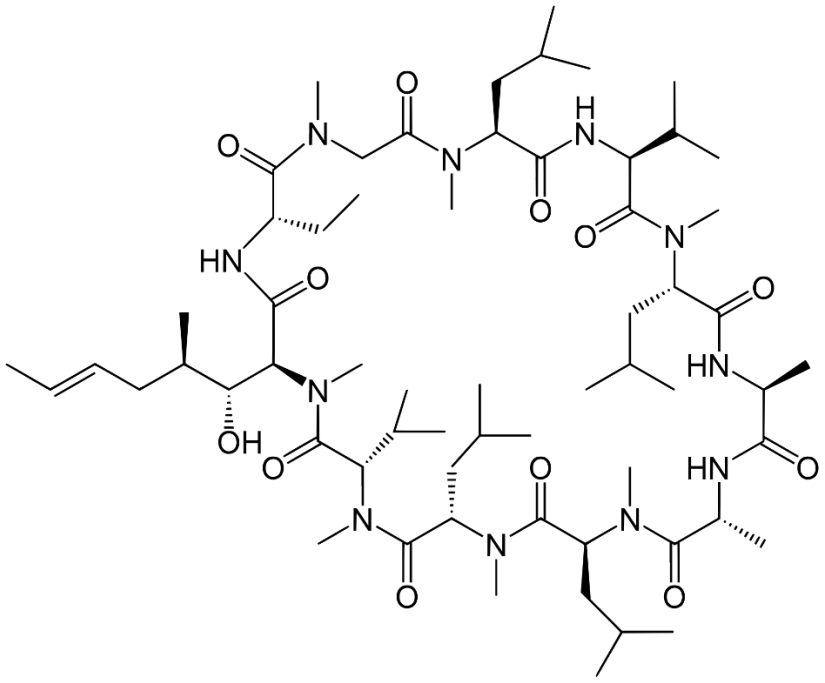

Figure 1. Structure of CsA (Borel et al. 1976)

CsA selectively inhibits the production of lymphokines, which are induced if T cells are activated. One of these lymphokines is interleukin-2, whose suppression results in a reduction of lymphocytes. CsA forms a complex with cyclophilin, a cytoplasmatic receptor protein. This complex inhibits calcineurin, an important phosphatase, and blocks antigen-triggered signal transduction in T lymphocytes. The consequence is an inhibition of transcription factors like nuclear factor of activated T cells (NFAT) or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Those transcription factors would be necessary to induce synthesis of cytokines in T lymphocytes. Since NFAT is found almost

exclusively in T lymphocytes, CsA is a very selective drug. Thus, it suppresses mostly cellular immunity and does not affect antibody synthesis. This makes it an excellent drug against transplant rejection and some autoimmune diseases (Krensky et al. 2011; Schild and Förstermann 2013; Wiederrecht et al. 1993).

CsA can be administered orally or intravenously, mostly suspended in 0.9 % NaCl (Fachinformation Sandimmun® 50 Mg/Ml). Since it is highly hydrophobic, it is made soluble by using ethanol-polyoxyethylated castor oil. If administered orally, only 20-50 % of the drug is absorbed and up to 30 % is inactivated when first passing the liver resulting in a wide inter- and intraindividual variation of bioavailability. Peak blood concentrations can be measured 1.5-2 hours after drug administration. The drug is eliminated from the blood in a biphasic manner with a terminal half-life of approximately 19 hours (range: 10-27 hours). CsA is distributed broadly outside the blood volume with app. 33-47 % in plasma, 41-58 % in erythrocytes, 5-12 % in granulocytes and 4-9 % in lymphocytes. A saturation in the uptake of leukocytes and erythrocytes occurs if CsA concentrations are high. In plasma, approximately 90 % is bound to proteins, mainly lipoproteins. Only 0.1 % of CsA is excreted unchanged in urine. The drug is largely metabolized in the liver by cytochrome P450, family 3, subfamily A (CYP3A). Some metabolization also takes place in the gastrointestinal tract and the kidneys. The metabolites are less biologically active and less toxic. 25 such metabolites have been found in human bile, feces, blood and urine. CsA is eliminated primarily through the bile into the feces. Patients with hepatic dysfunction thus have the need for adjusted dosage (Krensky et al. 2011; Schild and Förstermann 2013; Schreiber and Crabtree 1992; Transplants by Donor Type 2015).

CsA is nephrotoxic, hepatotoxic and neurotoxic. Hypertension, tremor, hirsutism, hyperlipidemia, diabetes, malignancy and gum hyperplasia are frequent adverse effects. In rare cases, CNIs can induce life-threatening thrombotic thrombocytopenic purpura / hemolytic uremic syndrome (TTP/HUS; cf. 6.4.); (Atkinson et al. 1984; Barbarino et al. 2013; Dlott et al. 2004; Gluckman et al. 1981; Krensky et al. 2011).

2.1.2. Tacrolimus

Tac (also referred to as FK-506) is a macrolide, produced by *Streptomyces tsukubaensis*. It was first described in Japan in 1987 (Kino et al. 1987) and tested in patients for the first time in 1989 (Starzl et al. 1989). Tac is 10- to a 100-fold more potent than CsA (Wiederrecht et al. 1993). Although CsA and Tac are chemically completely unrelated, they both have the same mode of action in cells (Wiederrecht et al. 1993). Tac binds to an intracellular immunophilin called FK506-binding protein-12 (FKBP-12). Subsequently, calcineurin phosphatase activity is inhibited by a complex of tracrolimus-FKBP-12, calcium, calmodulin and calcineurin. Therefore, CsA and Tac are both considered calcineurin inhibitors (CNIs) targeting the same pathway for immunosuppression (Krensky et al. 2011).

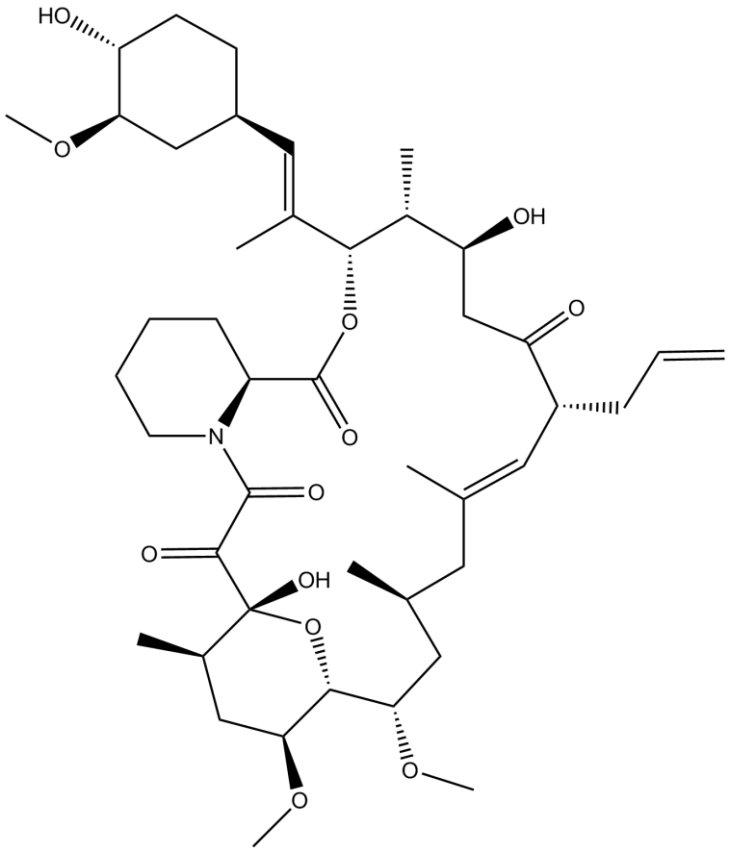

Figure 2: Structure of Tacrolimus (Kino et al. 1987)

Similar to CsA, Tac can be prescribed orally or intravenously. If administered into the vein, it is usually diluted in 0.9 % NaCl (Fachinformation Prograf 5 Mg/Ml Konzentrat Astellas.). Oral bioavailability is very variable ranging from 5-56 %. Plasma protein binding of Tac is approximately 99 % - mainly to albumin and alpha-1-acid glycoprotein -, regardless of its concentration. In whole blood, Tac is essentially bound to erythrocytes resulting in a ratio of Tac concentration in whole blood to Tac concentration in plasma of an average of 35 (range: 12 to 67). The half-life of Tac in healthy volunteers after intravenous (i.v.) administration yields 34 h but can come down to 12 h in patients after liver transplantation. Tac is mainly metabolized in the liver by the cytochrome P450-3A4 system and only less than 1 % of the intravenously dose administered is excreted unchanged in urine. Fecal elimination, on the other hand, accounts for 92 % (Astellas Prograf U.S. Physician Prescribing Information. 2015; Schild and Förstermann 2013).

Similar to CsA, Tac has multiple side effects including nephrotoxicity. Alopecia, tremors and new-onset diabetes mellitus, however, are more likely to be developed if patients are treated with Tac compared to CsA (Barbarino et al. 2013; Pirsch et al. 1997; Tricot et al. 2005; Williams and Haragsim 2006).

2.2. Central Venous Catheters

Every year, over 5 million central venous catheters (CVCs) are placed by physicians in the USA alone (Frasca et al. 2010). A CVC is defined as a catheter that is inserted into a great vessel whose tips rest in the superior vena cava or the right atrium (Rupp et al. 2012). It is most commonly placed in the subclavian vein, the internal jugular vein or the femoral vein, depending on the patient's anatomy, the risk associated with the placement and the skills of the operator (McGee and Gould 2003; Timsit 2002). Patients who require a CVC usually are critically ill and administration of volume, drugs and parenteral nutrition is ensured by placing a central line (McGee et al. 1993).

Since its experimental introduction into medicine in 1952 (Aubaniac 1952), many types of CVCs have been developed. The ones most frequently used are non-tunneled CVCs which are inserted directly percutaneously into a great vein. Tunneled CVCs, on the other hand, are passed under the skin from the insertion site to a separate percutaneous exit. Totally implantable catheters have a subcutaneous port which can be accessed with a needle. Peripherally inserted central venous catheters (PICC) are being placed into a smaller vein like the basilic, cephalic, or brachial vein but do enter the superior vena cava (O'Grady et al. 2011). In the following, CVC will always stand for the most commonly used CVCs, the non-tunneled ones.

CVCs are available with only one lumen or multiple lumina. Every lumen acts like an individual tube within the catheter with a separate exit at the end of the CVC. A CVC with multiple lumen allows staff to administer infusion of incompatible medications and fluids simultaneously and may therefore be adjusted to the patients' needs. Moreover, blood specimen can easily be taken through a CVC which avoids frequently disturbing the patient by multiple and painful blood withdrawals from peripheral veins (Gallieni et al. 2008).

Today, CVCs are made of a vast variety of different materials. They range from polyurethane (PU), polyethylene, polytetrafluoroethylene and polyvinyl chloride (PVC) to silicone. Catheter-related infections are very dangerous, especially to patients under immunosuppression. Therefore, in order to decrease infection rates, antiseptic components can be added to the material. The additives include chlorhexidine/sulfadiazine, rifampicin/minocycline and silver (Gallieni et al. 2008; Ramritu et al. 2008; Schiffer et al. 2013).

2.3. Therapeutic Drug Monitoring

Administering drugs is one of the major duties of physicians. For some drugs, the absorption and the metabolism are of great intraindividual and interindividual variety. Therefore, monitoring the concentration of the drug in the patient's blood is essential in order to

ensure that the levels remain within the narrow therapeutic window. Therapeutic drug monitoring (TDM) is applied if the therapeutic effect of a drug needs to be monitored by measuring its concentration in the patient's blood. This is often the case if the effects of a drug are not clinically obvious (e.g. hypertension) but more complex (e.g. immunosuppression). It is used in routine not only in the cases of CsA and Tac but also with many other drugs including cardiac glycosides, neuropsychiatric drugs and antibiotics (Fernandez et al. 2010; Hallbach 2011; Schiff et al. 2007).

Following a first oral dose of a CNI, peak levels (C_{max}) can be measured after 1 to 2 hours. After 2-6 days for CsA and 3-5 days for Tac, steady states are reached. Blood samples of CNI are usually collected immediately before the next dose is administered, thus obtaining trough levels (C_0) (Fernandez et al. 2010; Halloran 2004; Lee and Gabardi 2012; Schiff et al. 2007).

For CNIs, therapeutic drug monitoring (TDM) is especially reasonable since they have a narrow therapeutic range and there is a clear correlation between the concentration on the one hand and effects or clinical outcomes of the drugs on the other hand. In knowing the actual concentration of the drug in the patient's blood, physicians can easily adjust the dosage in order to ensure optimal and individualized immunosuppression for a patient. (Hogan and Storb 2004; Larkins and Matsell 2014; Wallemacq 2004)

3. Problem

Falsely elevated drug concentrations of CNIs (most often CsA; rarely Tac) in patients with CVCs have repeatedly been reported to be caused by reversible adsorption of the drugs to the CVC (Bleyzac 2013; Blifeld and Ettenger 1987; Busca et al. 1994; Carreras et al. 1988; Claviez et al. 2002; de Witte et al. 1986; Duffner et al. 1998; Fitzsimmons and Ponzillo 1988; Grouzmann et al. 2008; Hacker et al. 2011; Hacker et al. 2014; Leson et al. 1989; Lorenz et al. 1991; Shaefer et al. 1993). When infused through a CVC, the drug strongly adsorbs to the inner surface of the catheter. If blood is withdrawn from the same lumen, the drug is released again, resulting in spuriously high concentrations in the blood sample.

Usually, the falsely elevated concentrations are extremely high, immediately resulting in further analysis. The medical personnel in the laboratory would repeat the measurements and verify the high concentration. If the concentrations continue to prove unreasonably high, there will be careful investigations of the source of error. In most of these cases, the error simply emerges from wrong timing of the blood withdrawal. Thus, unreasonably elevated drug concentrations are deemed incorrect, not resulting in any further dosage adjustments or any harm for the patient.

One scenario, on the other hand, can be extremely critical for the patient: if the elevated concentration still falls into the upper reference range, the false elevation may remain undetected. Since the spurious concentration is not extremely elevated but only slightly raised, it might not lead to any further investigation but is misleadingly deemed correct. If so, the falsely elevated CNI concentrations can result in dangerous dose reduction of the immunosuppressive drug. This can put the patient at risk of life-threatening underdosage which may even cause organ rejection or GVHD (Geissler and Schlitt 2009; Ram et al. 2012).

In 2007, a case at the Klinikum rechts der Isar, Munich, (cf. patient A) shed light on this pitfall (Schneider et al. 2007): the patient had been switched to oral Tac medication two days before a blood sample was accidentally taken from the lumen previously used for infusing the drug. The concentration resulted in a toxic value of 86 µg/L of Tac. However, the control

sample obtained by venipuncture showed a level as low as 2.9 µg/L of Tac. This striking discrepancy was the trigger to perform a comprehensive in vitro and in vivo study on this effect (Hacker et al. 2014).

To systematically understand this adsorption and release effect for CsA and Tac in vitro, CVC systems most widely used in the US and Europe were examined. 30 CVCs were analyzed, consisting of different materials, namely polyurethane (PU), silicone and PU with an incorporated silver ion-based antimicrobial agent.

In addition to the comprehensive in vitro experiments, a prospective in vivo study was conducted with fifteen patients receiving CsA or Tac via a CVC after allogeneic stem cell transplantation.

The aim of the study was to quantify the reversible adsorption of CNIs to CVCs in vitro as well as in vivo and to evolve practical advice as to whether blood sampling for therapeutic drug monitoring can be performed via CVCs.

4. Materials and Methods

4.1. In Vitro Experiments

4.1.1. Basic Study

Four types of catheters, made of three different materials, were examined in vitro: Arrow-Howes™ “Quad-Lumen Central Venous Catheterization Set” (n = 6, PU-AH-CVC), Intra “Trilucath” (n = 2, PU-INCVC), Vygon “Lifecath Apheresis Plus” (n = 2, silicone-Vy-CVC) and Vygon “Multicath 4 Expert” (n = 2, PU with an incorporated silver ion-based antimicrobial agent, silver-Vy-CVC; cf. table 1 and 11.2.: images 1-4) (Hacker et al. 2014).

One of the following doses was infused into one lumen of a CVC to mimic a therapeutic in vivo application of the drug: 250 mg of CsA (n = 10; Sandimmun, Novartis Pharma) in 100 mL NaCl solution (0.9 %) (2.5 g/L of CsA) over 6 h or 2 mg of Tac (n = 10; Prograf, Astellas) in 50 mL NaCl (40 mg/L of Tac) over 22 h. Right after infusing the drug, blood sampling was mimicked in all four lumina with 4 mL of Fresh Frozen Plasma (FFP) each, always discarding the first 2 mL. When the drugs were infused and blood sampling was simulated, special attention was given to avoid cross contamination between the different lumina, particularly at the exits of the individual ports at the distal end of the catheter. Therefore, FFP was infused retrogradely with a syringe and collected at the distal end without getting in contact with the exits of the other lumina (cf. 11.2., images 5, 6 and 7) (Hacker et al. 2014) .

The CVC then was recurrently rinsed with NaCl (0.9 %), the first volume always being 10 mL, followed by multiple flushes of 1 L of NaCl. Each rinsing was followed by mimicking a blood withdrawal with 12 mL of FFP. To thoroughly analyze the withdrawal, the 12 mL were collected in six separate 2 mL doses (P0 – P5; cf. 11.2., image 7). Out of the six samples, the first 2 mL (P0) were always discarded in case they might be contaminated with fluids

previously administered through the lumen. The lumina of the CVCs were not blocked with any fluid at any time (Hacker et al. 2014).

Abbreviation	**Name**	**Material**	**Brand**	**Number of Lumina**	**Inner Diameter of Contaminated Lumen (mm)**	**Total Length of Contaminated Lumen (cm)**
PU-AH-CVC	Quad-Lumen Central Venous Catheterization Set	Polyurethane	Arrow-Howes™, (Kernen, Germany)	4	1.52	46.6
PU-In-CVC	Trilucath	Polyurethane	Intra, (Rehlingen-Siersburg, Germany)	3	0.80	36.5
silicone-Vy-CVC	Lifecath Apheresis Plus	Silicone	Vygon (Aachen, Germany)	3	1.90	50.0
silver-Vy-CVC	Multicath 4 Expert	Polyurethane incorporated with silver ion-based antimicrobial agent	Vygon, (Aachen, Germany)	4	1.25	35.8

Table 1: CVCs used in in vitro and in vivo experiments (cf. 11.2., images 1-4) (Hacker et al. 2014).

4.1.2. Water Bath

In eight additional cases, infusing the drug was performed in a water bath at 37°C in order to create a more realistic setting. This way, it was possible to compare the results measured at

room temperature to experiments conducted at body temperature. A set of four CVCs each consisting of one of the tested CVCs (cf. table 1) was infused with CsA. An identical set of four CVCs was infused with Tac (Hacker et al. 2014).

4.1.3. EDTA Blood

In addition to the 20 experiments conducted with FFP only, six PU-AH-CVCs were used for further investigations. This time, some mimicked blood sampling was performed with uncontaminated whole blood instead of FFP. The blood was taken into tubes containing ethylenediaminetetraacetic acid (EDTA). The aim was to study whether whole blood and FFP behave similarly:

Four CVCs were investigated in the usual manner, except for mimicking the blood collection alternating with FFP and EDTA blood. These experiments were performed with both drugs once at room temperature and once with the drug being infused in a water bath at 37°C.

To study possible adsorption effects with the drugs' concentration being in the therapeutic range, two PU-AH-CVCs were put into EDTA blood for 24 h with a concentration of 125 µg/L of CsA (n = 1) and 10 µg/L of Tac (n = 1) respectively. Blood sampling was mimicked with EDTA blood after rinsing the CVCs with 10 mL of NaCl (Hacker et al. 2014).

4.1.4. Fat Emulsion

In order to examine the effect of oily fluid on the release of the drugs, two PU-AH-CVCs were infused with CsA and Tac respectively. After flushing them with 1.01 L of NaCl, 100 mL of fat emulsion (ClinOleic 20 %, Baxter) was infused. Blood sampling was then mimicked with 3 x 2 mL of EDTA blood, discarding the first 2 mL. Another 1.01 L of NaCl and 50 mL of fat emulsion was infused into the CVC previously contaminated with Tac. Afterwards, blood sampling was performed in the same manner as just described above (Hacker et al. 2014).

4.1.5. Anterograde vs. Retrograde Sampling

To study the effect of mimicking most of the in vitro blood sampling retrogradely with a syringe (like infusing something in vivo), a set of special experiments was performed. Four PU-AH-CVCs were infused immunosuppressants: two of them with CsA and two with Tac respectively. After rinsing the CVCs with 3 L of NaCl as well as 20 mL of FFP, blood sampling was mimicked with FFP. This time, withdrawing FFP was performed retrogradely as well as anterogradely in alternation. Each time, the first 2 mL (P0) of the sample were discarded while the following 2 mL (P1) were used for measurement. While performing the experiment the CVCs were not rinsed with NaCl.

4.2. In Vivo Study

A monocentric, prospective pilot study was conducted, approved by the ethics committee of the Klinikum rechts der Isar, Munich, and in accordance with the current revision of the Helsinki Declaration. Fifteen in-patients after allogenic stem cell transplantation aged 22 to 68 were included after giving informed consent (patient 1-15; cf. table 8; cf. information and consent forms 11.3.). Twelve of them were receiving CsA and three were treated with Tac.

In all cases, blood withdrawal was performed in all lumina of the CVC, each time discarding the first 5 mL of blood as it is clinical practice at the Klinikum rechts der Isar. Additionally, a blood sample was taken simultaneously via peripheral venipuncture each time. All samples were collected into EDTA containing tubes of 2.7 mL (Hacker et al. 2014).

Patient A was not part of the official in vivo study since the case had already happened in 2007 entailing the initiation of the in vitro as well as the in vivo study later on.

4.2.1. Main Study

In thirteen cases (patient 1-13, cf. table 8), the drug had been administered via a PU-AH-CVC (cf. table 1). Doses of 75 mg to 750 mg of CsA and 1.0 mg to 2.5 mg of Tac had been administered over 7 to 22 days. The drug was received each day for 22 h, followed by flushing with 50 mL of NaCl for two hours. Trough blood samples for the study were taken after the two hours of rinsing, just before the next dose of CNIs was administered (Hacker et al. 2014).

4.2.2. Special Cases

The only distinction in patient 14 was the fact that CsA administration had been switched to oral medication three days prior to blood sampling.

Patient 15 had received only a single dose of Tac via a silver-Vy-CVC (cf. table 1). Due to high Tac concentrations in the blood, the infusion with a concentration of 60 mg/L of Tac was stopped after 40 mL had been administered. For the following three days, Tac administration was stopped completely. On the third day after withdrawing Tac administration, blood samples were taken for the study.

Patient A had received Tac for 27 days via a three-lumen CVC made of polyurethane by Arrow-Howes™. Blood samples were taken on day 7, 8 and 18 after stopping Tac administration through the CVC (Hacker et al. 2014).

4.3. Measuring

All in vivo and in vitro samples were measured in duplicate by a liquid chromatography/tandem mass spectrometry (LC/MS/MS) immunosuppressant method modified from Koal et al. (Koal et al. 2005) and Ceglarek et al. (Ceglarek et al. 2004) using a two dimensional high performance liquid chromatography (HPLC) system coupled to an API

3000 tandem mass spectrometer. Mobile phase chemicals were purchased from Sigma-Aldrich, controls from Chromsystems Instruments & Chemicals and standards from Sigma-Aldrich, Chromsystems Instruments & Chemicals and Recipe Chemicals + Instruments.

Samples were precipitated by a $ZnSO_4$/methanol mix containing the internal standards. The analytes were captured by a Cyclone® HTLC column and separated by a phenyl-hexyl column before analyzed in the mass spectrometer. Accuracy was ensured by continuously participating in proficiency testing schemes. If not measured within the first 6 h after it was obtained, each sample was kept at 8°C and analyzed within a maximum of 72 h. For longtime storage, all samples from the in vitro and in vivo experiments were frozen at -25°C (Hacker et al. 2014).

4.4. Calculations

All in vitro data were normalized with regard to the total inner surface of the analyzed lumen using PU-AH-CVC as reference.

Statistical analyses of the in vitro experiments were performed using the Wilcoxon signed rank test. A two-sided level of significance of 5 % was used (Hacker et al. 2014).

5. Results

5.1. In Vitro Experiments

Regardless of the material, every CVC which had been in contact with high concentrations of the drug used in the infusion showed significant evidence of reversible adsorption for CsA (n = 13, p = 0.001) and Tac (n = 13, p = 0.001, Wilcoxon signed rank test) (Hacker et al. 2014).

5.1.1. Basic Study

Immediately after infusing the drugs and discarding only the first 2 mL of FFP, mean concentrations of 6420 µg/L of CsA (n = 12) and 250 µg/L (n = 12) of Tac were measured. The maximum concentrations yielded 17700 µg/L for CsA and 395 µg/L for Tac. These values are about 100 times lower than the usual concentration of the solution infused into a patient (e.g. 2.5 g/L of CsA, 40 mg/L of Tac; cf. 4.1.1.). The lowest concentrations measured during the first mimicked blood sampling were 260 µg/L for CsA and 93 µg/L for Tac (cf. figure 3 and 4) (Hacker et al. 2014).

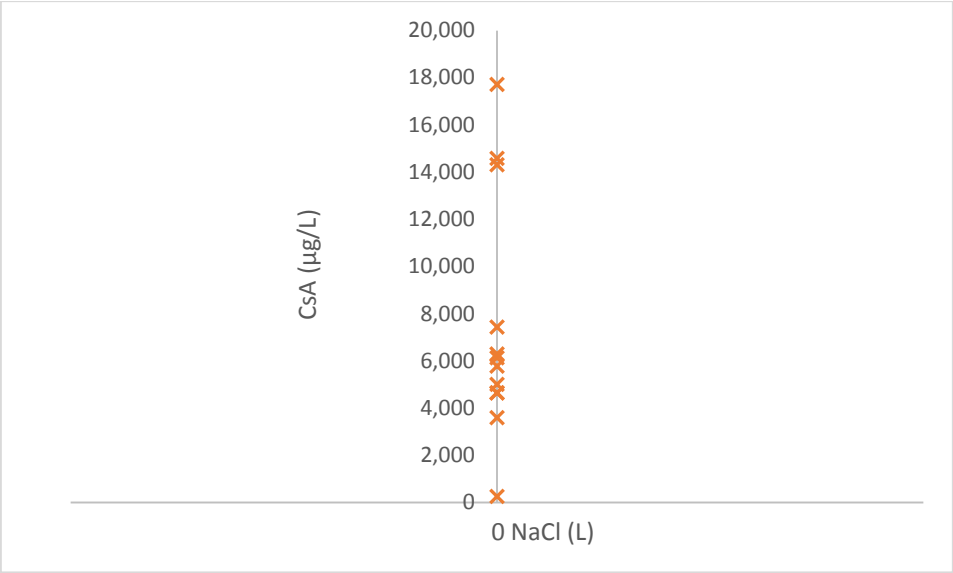

Figure 3: Basic in vitro study: concentration of CsA in first mimicked blood sampling immediately after CsA infusion (n = 12: 6 x PU-AH-CVC, 2 x PU-In-CVC, 2 x silicone-Vy-CVC, 2 x silver-Vy-CVC). 2 mL of FFP discarded prior to sampling.

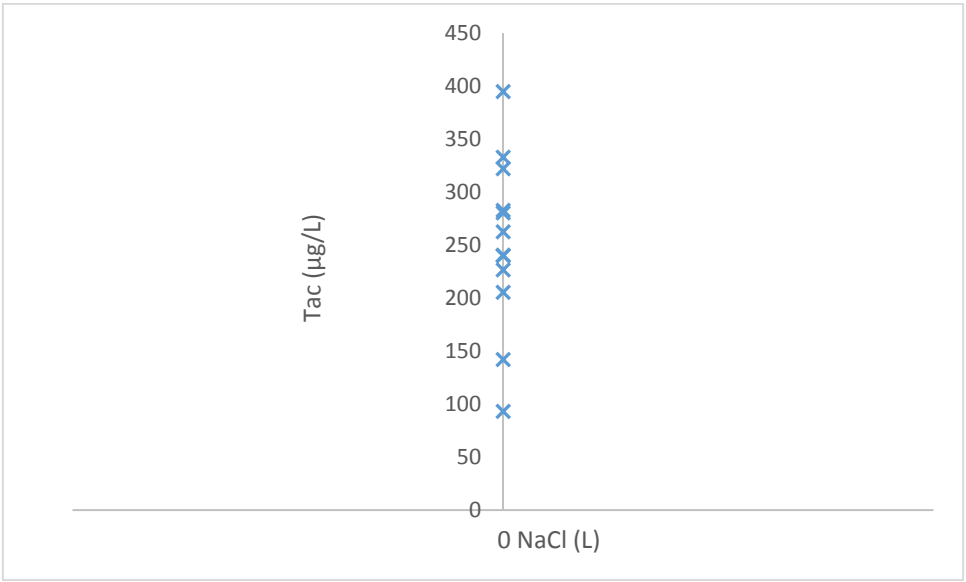

Figure 4: Basic in vitro study: concentration of Tac in first mimicked blood sampling immediately after Tac infusion (n = 12: 6 x PU-AH-CVC, 2 x PU-In-CVC, 2 x silicone-Vy-CVC, 2 x silver-Vy-CVC). 2 mL of FFP discarded prior to sampling.

After the first mimicked blood sampling, the CVC was rinsed with 10 mL of NaCl followed by sampling 6 portions of 2 mL of FFP. The first 2 mL (P0) were always discarded. The mean concentrations measured in the first portion (P1) yielded 132 µg/L for CsA (n = 6) and 55 µg/L for Tac (n = 6) and decreased to 115 µg/L for CsA and 36 µg/L for Tac in the last portion (P5) (Hacker et al. 2014).

Rinsing the lumen with several liters of NaCl resulted in further reductions of the drug concentration when blood sampling was mimicked. After discarding 6 mL each time, CsA and Tac values diminished as shown in table 2.

L of NaCl used for rinsing	**Decline of mean value for CsA**	**Decline of mean value for Tac**
1.01	- 57 % (n = 7*)	- 52 % (n = 8*)
2.01	- 22 % (n = 8)	- 42 % (n = 10)
3.01	- 41 % (n = 8)	- 38 % (n = 10)
4.01	- 42 % (n = 8)	- 3 % (n = 10)
5.01	- 19 % (n = 7*)	- 3 % (n = 10)

Table 2: Decline of mean value per L of NaCl. First 6 mL always discarded.
** Varying numbers of sampling points due to lack of performed measurement step in certain CVCs.*

However, even after extensive rinsing with NaCl, drug release was still detectable in many cases. The maximum volume of NaCl administered after which drug release was still measurable differed between the different types of CVCs:

Type of CVC	Maximum volume of NaCl (L)	CsA release (µg/L)
PU-AH-CVC	7.01	35
silicone-Vy-CVC	13.01	23
silver-Vy-CVC	5.01	6

Table 3. Maximum volumes of NaCl used to rinse catheters which still produced CsA release when blood sampling was mimicked with FFP. First 6 mL of FFP discarded.

Type of CVC	Maximum volume of NaCl (L)	Tac release (µg/L)
PU-AH-CVC	15.01	2.2
silicone-Vy-CVC	24.01	4.4
silver-Vy-CVC	11.01	2.0

Table 4. Maximum volumes of NaCl used to rinse catheters which still produced Tac release when blood sampling was mimicked with FFP. First 6 mL of FFP discarded.

5.1.2. Different Materials

All three materials adsorbed and released the drugs throughout the entire study. The effect was strongest in CVCs made of silicone compared to PU and silver (cf. figure 5) (Hacker et al. 2014).

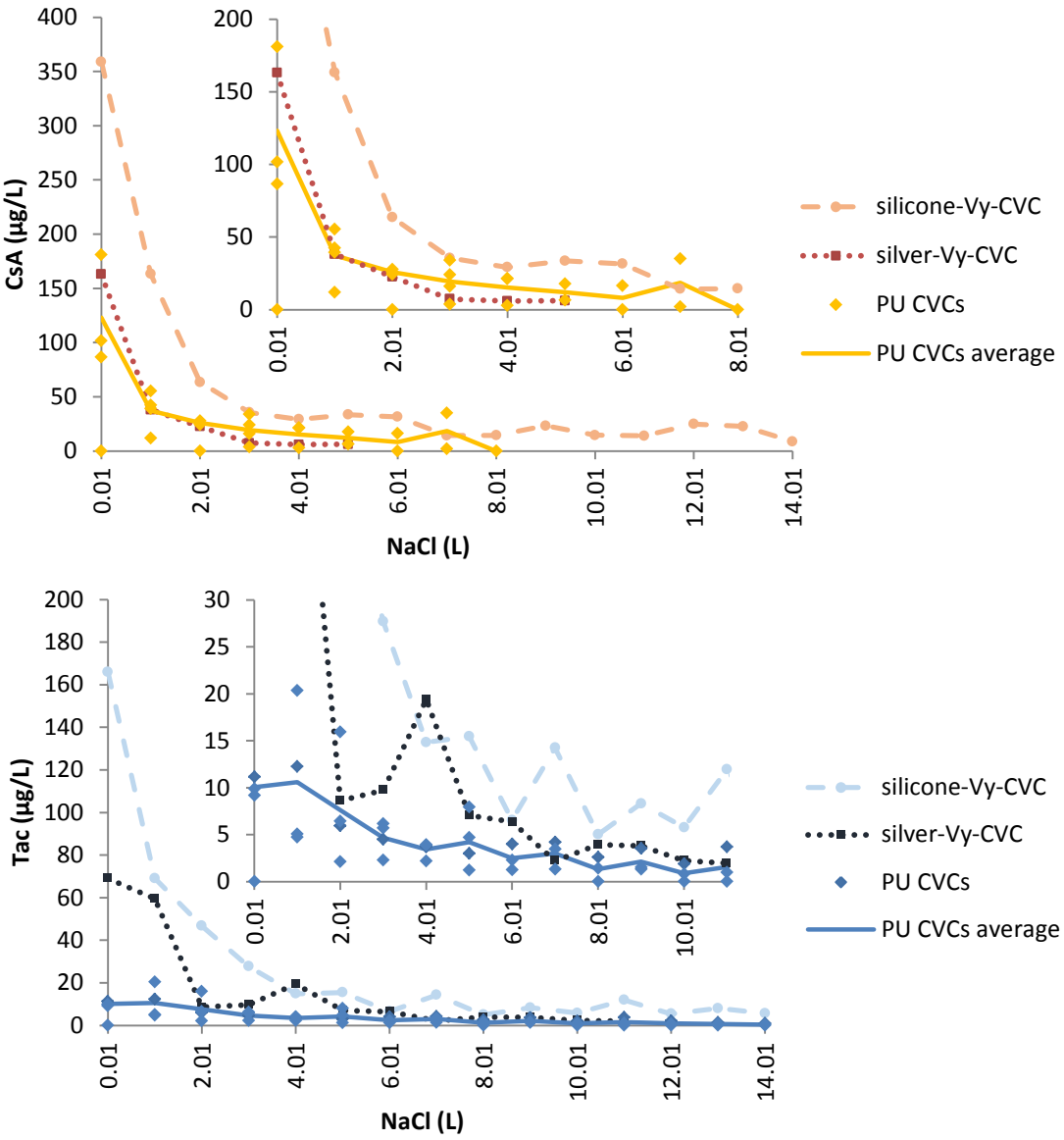

Figure 5. Drug concentrations from 12 CVCs: one set of 1 silicone-Vy-CVC, 1 silver-Vy-CVC and 3 PU CVCs (2 PU-AH-CVCs, 1 PU-In-CVC) infused with CsA and one set with Tac. P3 of blood sampling mimicked with FFP after rinsing multiple times with NaCl. Average line for PU enclosed. Part of the figure zoomed in and displayed in the upper right corner (Hacker et al. 2014).

The change of the drug concentration from portion 1 (P1) to portion 5 (P5) of each mimicked blood sampling varied significantly with the materials. In the case of CsA, the drug concentration declined by a mean value of 46 % (n = 26; range: -91 % to 3 %) for CVCs made of PU and a mean value of 63 % (n = 27; range: -88 % to -10 %) for silver-Vy-CVCs, respectively. The concentration measured with silicone-Vy-CVCs, however, increased by a mean value of 99 % (n = 20; range: -2 % to 271 %) from P1 to P5 (cf. figure 6).

The same disparity was shown for Tac: the values of the CVCs made of PU decreased by a mean value of 67 % (n = 74; range: -67 % to 19 %) as well as a mean value of 49 % (n = 33; range: -93 % to 35 %) in the cases of silver-Vy-CVCs. Per contra, the silicone-Vy-CVCs showed higher concentrations in P5 by a mean value of 42 % (n = 46; range: -65 % to 398 %) compared to P1 (cf. figure 7).

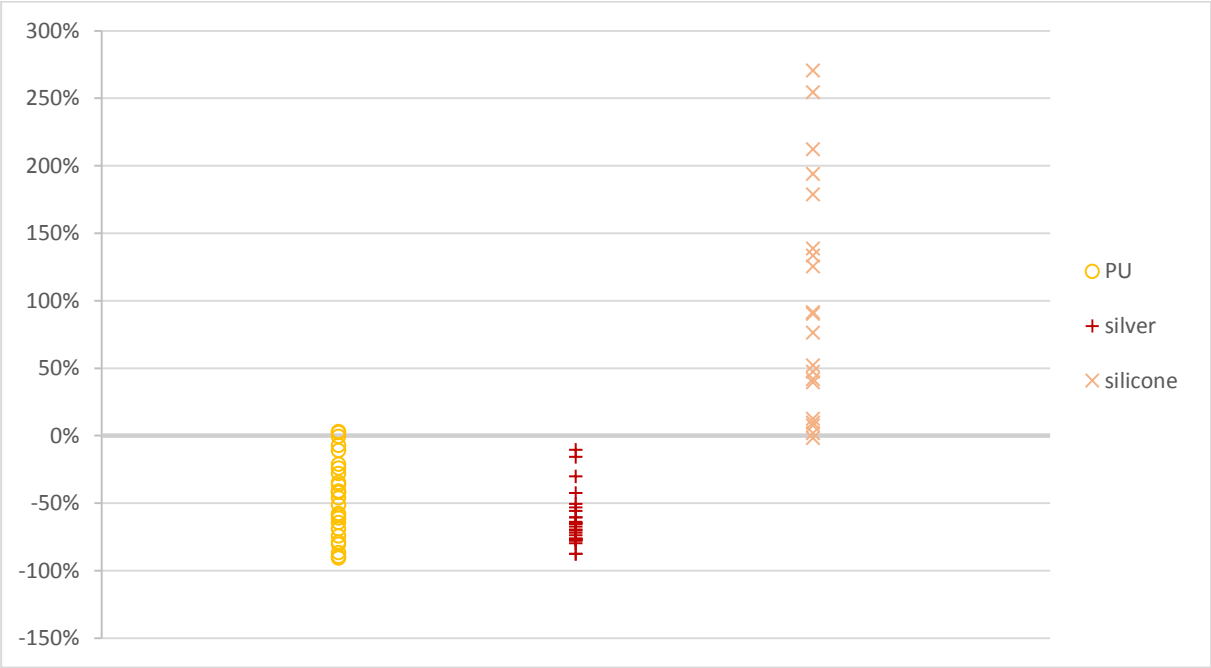

Figure 6. Change of CsA concentrations from P1 to P5 in percent.

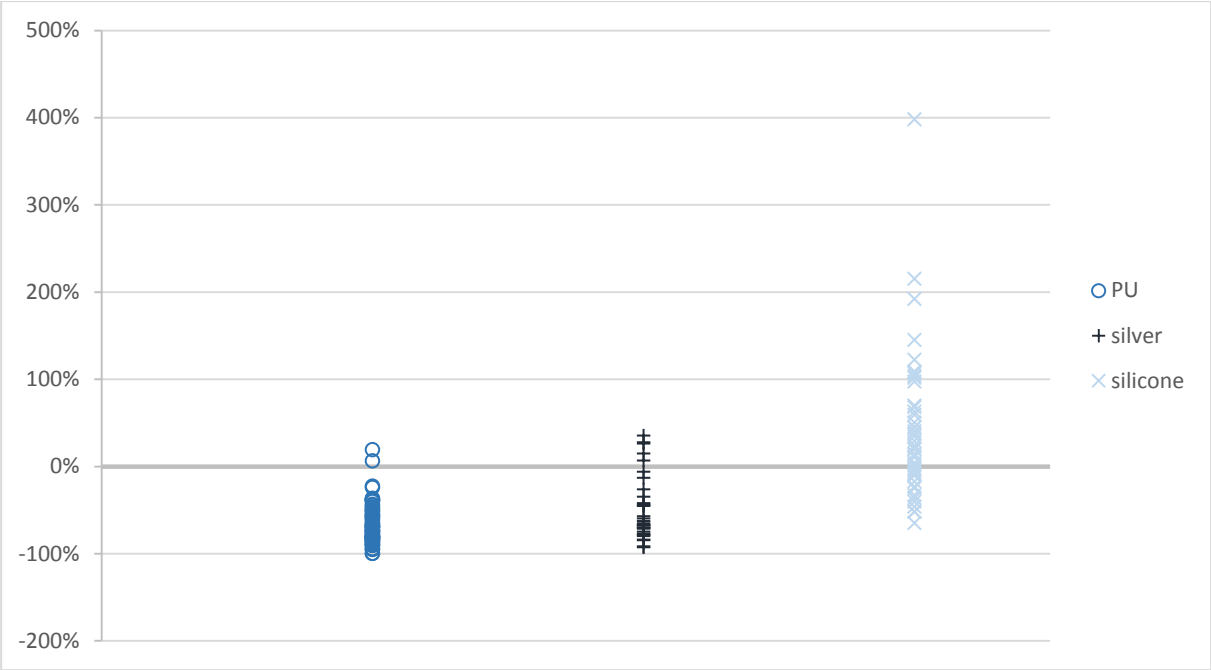

Figure 7. Change of Tac concentrations from P1 to P5 in percent.

Similar to the changes from P1 to P5, the difference from the last portion of a mimicked blood sample (P5) and the first portion of the following mimicked blood sampling (P1) were studied as well. Between the two sets of mimicked blood sampling, each CVC was rinsed with 1 L of NaCl. The first portion of each set (P0) was discarded as usual.

For CsA, the concentration from P5 to the following P1 was increased by a mean value of 99 % (n = 22; range: -77 % to 2930 %) for CVCs made of PU and 210 % (n = 25; range: -45 % to 1087 %) for silver-Vy-CVCs. However, in the case of silicone-Vy-CVCs, the CsA levels declined by a mean value of 52 % (n = 19; range: -88 % to -10 %) (cf. figure 8).

Tac behaved similarly with the concentrations increasing from P5 to P1 by a mean value of 397 % (n = 68; range: -45 % to 4814 %) if CVCs were made of PU and of 185 % (n = 31; range: -78 % to 1332 %) if silver-Vy-CVCs were used. The levels of the released Tac concentration also decreased by a mean value of 2 % (n = 44; range: 347 % to -87 %) from P5 to P1 in the case of silicone-Vy-CVCs (cf. figure 9).

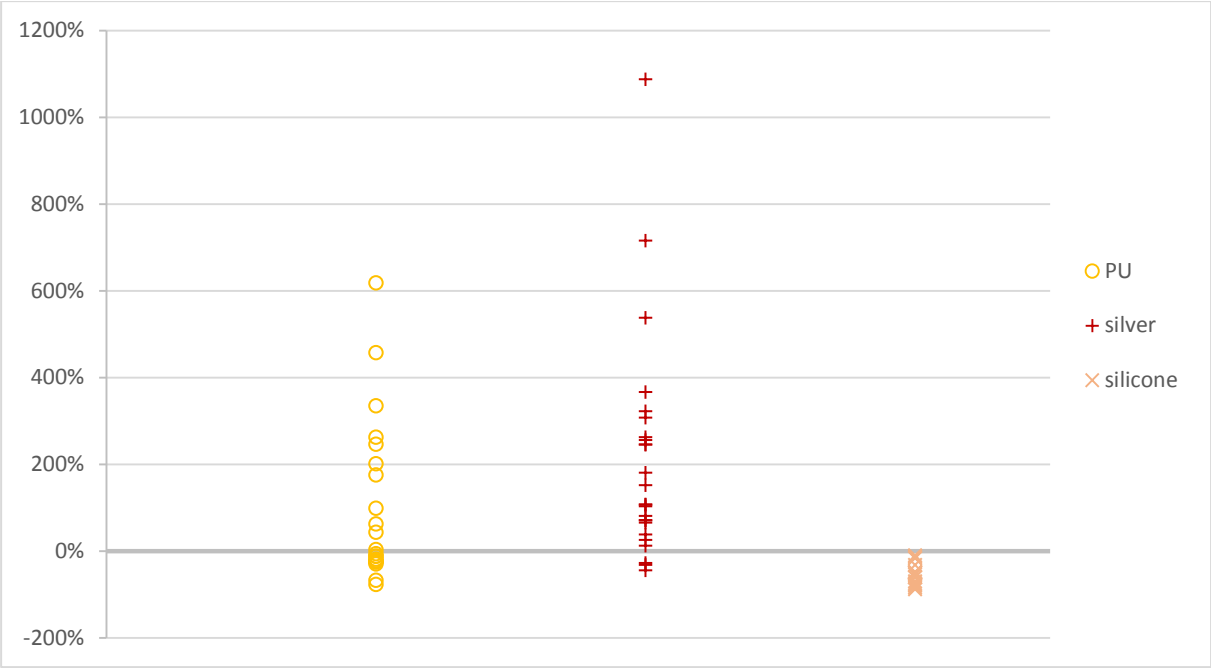

Figure 8. Change of CsA concentrations from P5 to following P1 in percent.

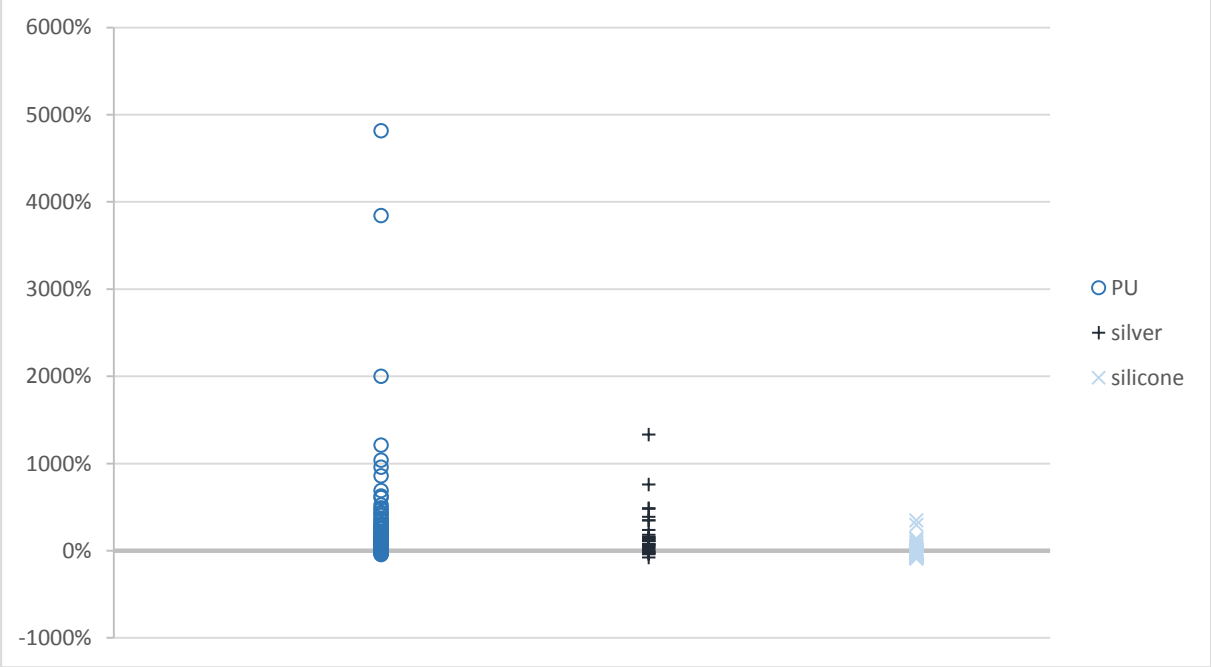

Figure 9. Change of Tac concentrations from P5 to following P1 in percent.

5.1.3. Water Bath

Performing the infusion of CsA and Tac in a water bath at 37°C resulted in higher concentrations of the drug when blood sampling was mimicked as opposed to infusing it at room temperature (cf. figure 10) (Hacker et al. 2014).

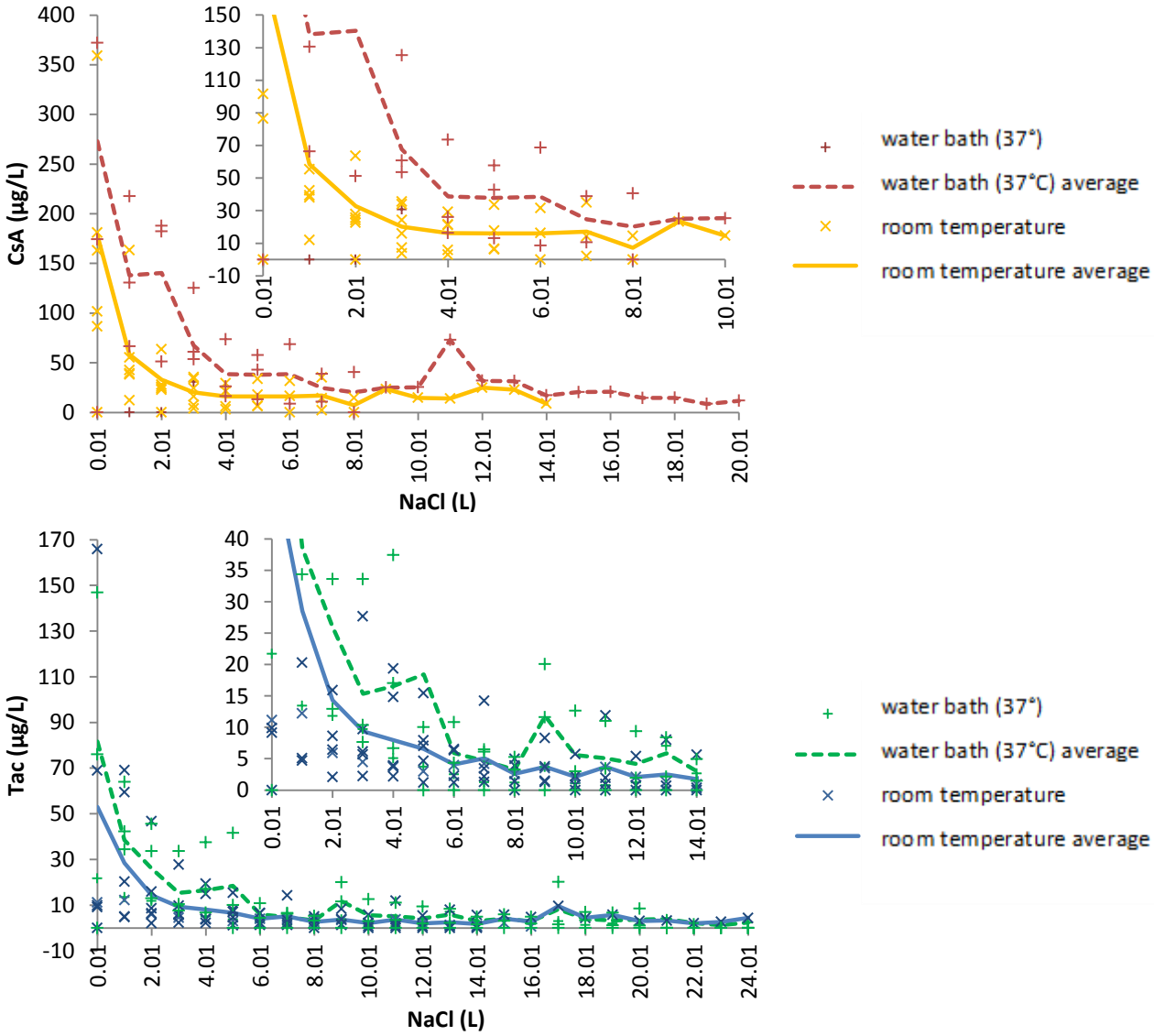

Figure 10. Drug concentrations from 20 CVCs: for CsA and Tac, 1 silicone-Vy-CVC, 1 silver-Vy-CVC, 1 PU-AH-CVC and 1 PU-In-CVC were infused in a water bath at 37°C. 1 silicone-Vy-CVC, 1 silver-Vy-CVC, 3 PU-AH-CVCs and 1 PU-In-CVC per drug were infused at room temperature. Blood sampling was mimicked with FFP after rinsing multiple times with NaCl. Each time, the first 6 mL of FFP were discarded. Average lines enclosed. Part of the figure zoomed in and displayed in the upper right corner (Hacker et al. 2014).

5.1.4. EDTA Blood

Performing the blood sampling with EDTA blood showed similar drug concentrations compared to performing it with FFP (cf. figure 11 and 12) (Hacker et al. 2014).

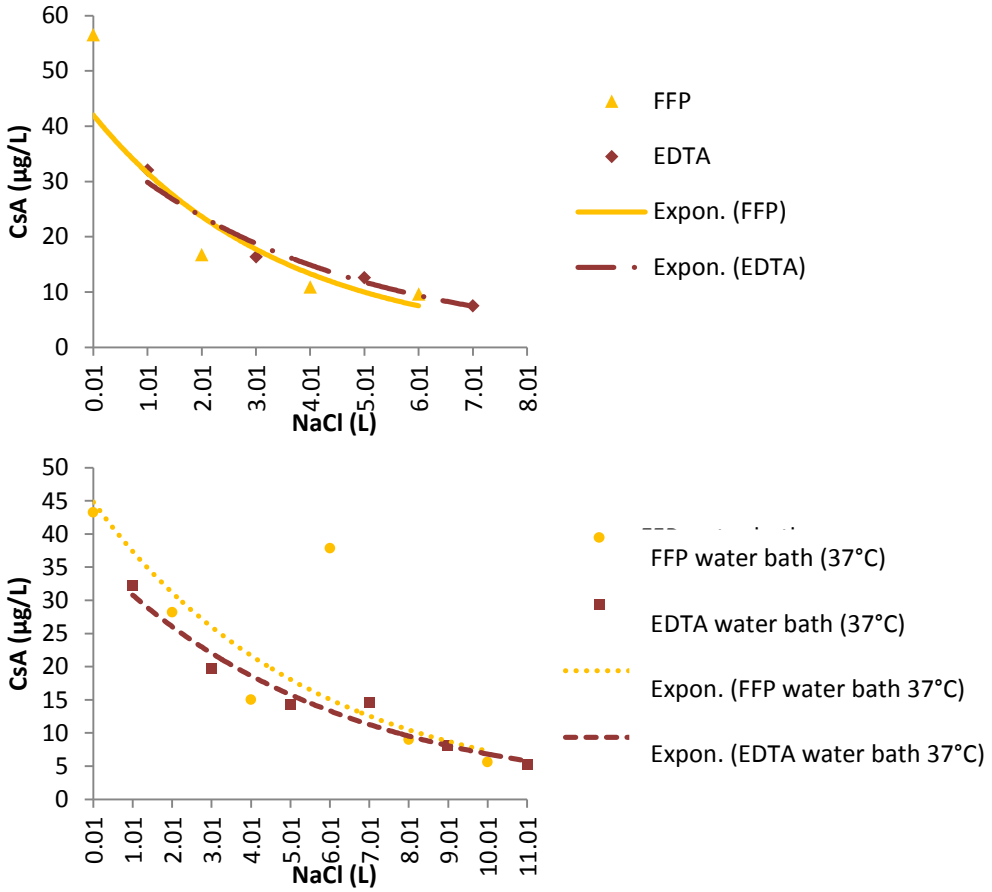

Figure 11. CsA concentrations from 2 PU-AH-CVCs, one of them infused in a water bath at 37°C. Blood sampling mimicked alternating FFP and EDTA blood after rinsing multiple times with NaCl. Each time, the first 6 mL of FFP and EDTA blood were discarded. Exponential fit line enclosed (Hacker et al. 2014).

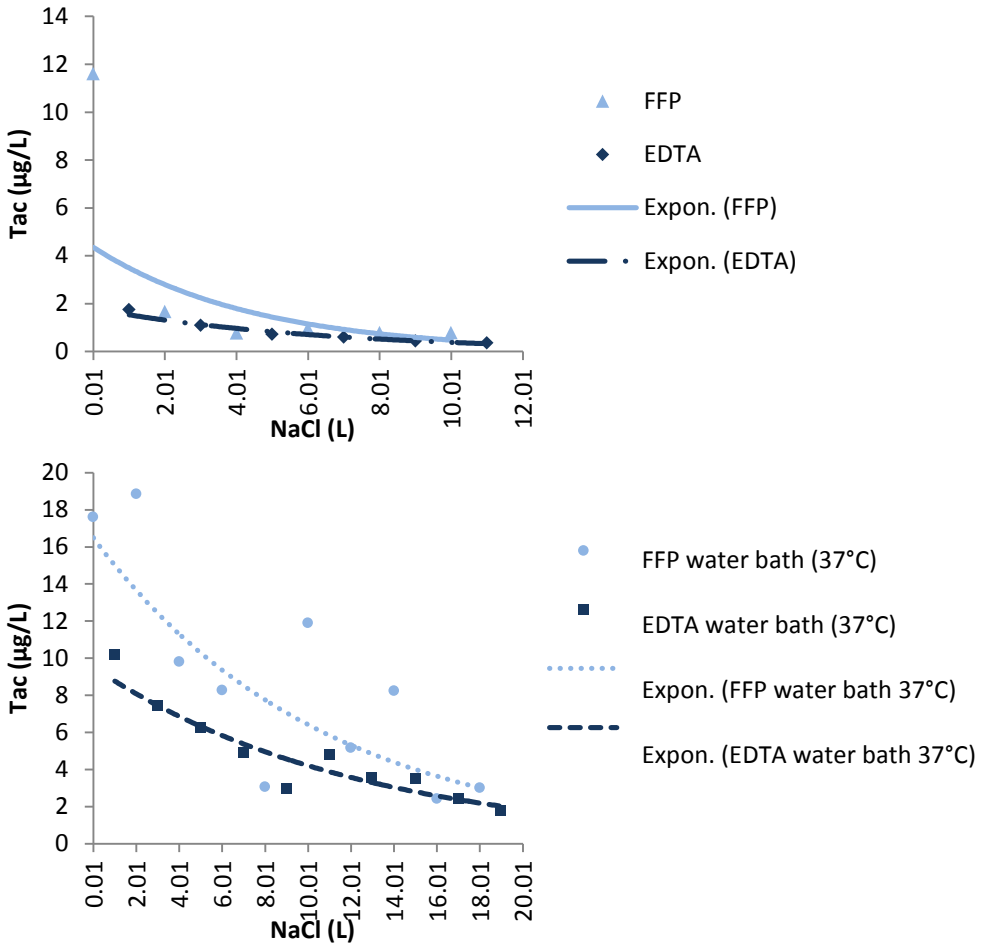

Figure 12. Tac concentrations from 2 PU-AH-CVCs, one of them infused in a water bath at 37°C. Blood sampling mimicked alternating FFP and EDTA blood after rinsing multiple times with NaCl. Each time, the first 6 mL of FFP and EDTA blood were discarded. Exponential fit line enclosed (Hacker et al. 2014).

The CVCs placed in EDTA blood containing low concentrations within the therapeutic range of CsA and Tac did not show any relevant reversible drug adsorption when blood sampling was mimicked (Hacker et al. 2014).

5.1.5. Fat Emulsion

After rinsing two PU-AH-CVCs with NaCl as well as fat emulsion, both CsA and Tac were still released when blood sampling was mimicked with FFP or EDTA blood (Hacker et al. 2014). However, the reversible adsorption was stronger in the case of Tac. After rinsing the CVCs with the same quantity of fat emulsion and NaCl, drug release was still detected in the CVC previously infused with Tac but not in the one infused with CsA, (cf. table 5 and 6).

Rinsing	**Mimicked blood sampling (first 2 ml (P0) always discarded)**
250 mg of CsA in 100 mL NaCl (2.5 g/L of CsA) for 6h	
10 ml of NaCl	
	2 ml of FFP (P1): 245 µg/L of CsA
1 L of NaCl	
	2ml of EDTA blood (P1): 20 µg/L of CsA
	2 ml of EDTA blood (P2) : 23 µg/L of CsA
100 ml of fat emulsion: ClinOleic 20 %, Baxter	
	2ml of EDTA blood (P1): 17 µg/L of CsA
	2 ml of EDTA blood (P2) : 11 µg/L of CsA
10 mL of NaCl	
	2ml of FFP (P1): 10 µg/L of CsA
	2 ml of FFP (P2) : 9 µg/L of CsA
	2ml of EDTA blood (P3): 3 µg/L of CsA
	2 ml of EDTA blood (P4) : 4 µg/L of CsA

Table 5. Rinsing and mimicking blood sampling of one PU-AH-CVC infused with CsA.

Rinsing	Mimicked blood sampling (first 2 ml (P0) always discarded)
2 mg of Tac in 50 mL NaCl (40 mg/L of Tac) for 22 h	
10 mL of NaCl	
	2 mL of FFP (P1): 36 µg/L of Tac
1 L of NaCl	
	2mL of EDTA blood (P1): 19 µg/L of Tac
	2 mL of EDTA blood (P2) : 14 µg/L of Tac
100 mL of fat emulsion: ClinOleic 20 %, Baxter	
	2mL of EDTA blood (P1): 17 µg/L of Tac
	2 mL of EDTA blood (P2) : 8 µg/L of Tac
10 mL of NaCl	
	2mL of EDTA blood (P1): 9 µg/L of Tac
	2 mL of EDTA blood (P2) : 10 µg/L of Tac
1 L of NaCl	
	2mL of EDTA blood (P1): 4 µg/L of Tac
	2 mL of EDTA blood (P2) : 4 µg/L of Tac
50 mL of fat emulsion: ClinOleic 20 %, Baxter	
	2mL of EDTA blood (P1): 5 µg/L of Tac
	2 mL of EDTA blood (P2) : 3 µg/L of Tac

Table 6. Rinsing and mimicking blood sampling of one PU-AH-CVC infused with Tac.

5.1.6. Anterograde vs. Retrograde Sampling

When blood sampling was mimicked retrogradely as well as anterogradely with a syringe, the absolute concentrations measured were comparable.

Performing two consecutive measurements with CsA in the range of 10 to 70 µg/L anterogradely or retrogradely, a similar reduction of 15 ± 9 % (n = 2) and 20 ± 13 % (n = 4) respectively can be observed.

Likewise, if performing two consecutive measurements with Tac in the range of 1 to 10 µg/L anterogradely or retrogradely, one obtains a similar reduction of 32 ± 0 % (n = 2) and 49 ± 13 % (n = 4) respectively.

	CsA (µg/L)	**CsA (µg/L)**	**Tac (µg/L)**	**Tac (µg/L)**
anterogradely	34.4	64.0	5.3	8.8
anterogradely	31.7	50.4	3.6	6.0
retrogradely	16.8	18.9	4.1	2.5
retrogradely	14.4	18.5	1.9	2.0
anterogradely	27.0	22.2	5.8	2.4
retrogradely	32.6	31.8	7.4	4.7
retrogradely	30.3	13.3	3.0	1.7
anterogradely	31.0	22.3	5.2	3.8

Table 7. Eight consecutive samples of FFP taken anterogradely or retrogradely. Fist 2 mL (P0) of each sample discarded.

5.2. In Vivo Study

For every patient, remarkably elevated concentrations of CsA and Tac were measured in the lumen previously used for infusing the drug (cf. table 8) (Hacker et al. 2014).

5.2.1. Main Study

In the eleven cases of CsA (patient 1-11; cf. table 8), the drug level obtained from the contaminated lumen was increased by a mean factor of 11.1 (range: 6.7 – 22.0) compared to the one acquired via venipuncture. The CsA concentrations from the samples obtained by venipuncture yielded a mean level of 191 µg/L (range: 96 – 249 µg/L) whereas the drug levels in the samples from the contaminated lumen resulted in a mean CsA concentration of 2120 µg/L (range: 1460 – 4410 µg/L). The blood samples from the lumina not used for infusing CsA showed results comparable (± 10 %) to the ones obtained by venipuncture (Hacker et al. 2014).

Patient 12 and 13 showed Tac concentrations raised by a factor of 150 and 116, respectively, in the lumen previously used for infusion (cf. table 8). The Tac level measured in the blood sample obtained by venipuncture resulted in 5.1 µg/L and 6.1 µg/L while the drug concentration in the sample from the contaminated lumen yielded 767 µg/L and 705 µg/L (Hacker et al. 2014).

In contrast to the results from all patients treated with CsA as well as patients 13 and 15, the blood sample of patient 12, from a lumen reportedly not used for infusing Tac previously, contained a 1.5 times elevated concentration of 7.6 µg/L of Tac compared to 5.1 µg/L obtained via venipuncture (Hacker et al. 2014). With a coefficient of variation of 8% for the concentration measured by the mass spectrometer, the factor of elevation results in a value of 1.5 ± 0.2 (confidence interval of 95%). Besides that fact that the measurement could represent an outlier, the possibility of a pre-analytical source of error leading to a single elevated drug concentration can never be ruled out.

	Drug	Type of CVC	Veni-puncture (µg/L)	Contami-nated Lumen (µg/L)	Lumen 2 (µg/L)	Lumen 3 (µg/L)	Lumen 4 (µg/L)	Duration of i.v. Drug applica-tion (days)	Current i.v. Drug Dosage (mg)	Time since last i.v. administra-tion (days)
Patient 1	CsA	PU-AH-CVC	235	2490	231	*)	277	8	300	-
Patient 2	CsA	PU-AH-CVC	96	2110	97	99	102	7	480	-
Patient 3	CsA	PU-AH-CVC	249	4410	257	275	*)	22	200	-
Patient 4	CsA	PU-AH-CVC	144	2255	138	143	147	10	750	-
Patient 5	CsA	PU-AH-CVC	172	1615	172	171	172	9	450	-
Patient 6	CsA	PU-AH-CVC	138	1460	147	*)	*)	13	75	-
Patient 7	CsA	PU-AH-CVC	192	1755	194	188	190	7	200	-
Patient 8	CsA	PU-AH-CVC	248	1655	254	265	249	16	400	-
Patient 9	CsA	PU-AH-CVC	199	1990	194	195	193	14	300	-
Patient 10	CsA	PU-AH-CVC	198	1635	201	201	200	15	500	-
Patient 11	CsA	PU-AH-CVC	229	1930	233	*)	238	16	200	-
Patient 12	Tac	PU-AH-CVC	5,1	767	4,9	7,6	5,5	21	2.0	-
Patient 13	Tac	PU-AH-CVC	6,1	705	6,0	6,4	6,2	11	1,75	-
Patient 14	CsA	PU-AH-CVC	296	895	292	295	286	22	-	3
Patient 15	Tac	Silver-Vy-CVC	12,9	37	13,5	13,4	13,6	1	-	3
Patient A	Tac	PU-AH-CVC ‡)	2.9	86	†)	†)	-	26	-	7
			4.6	171	5.9	3.3	-		-	8
			2.6	96	6.3	4.4	-		-	18

Table 8. Summary of data from all patients (Hacker et al. 2014).
**) Blood sampling was impossible due to blockage of the line.*
†) Blood sampling not performed.
‡) Arrow-Howes™ polyurethane CVC with three lumina.

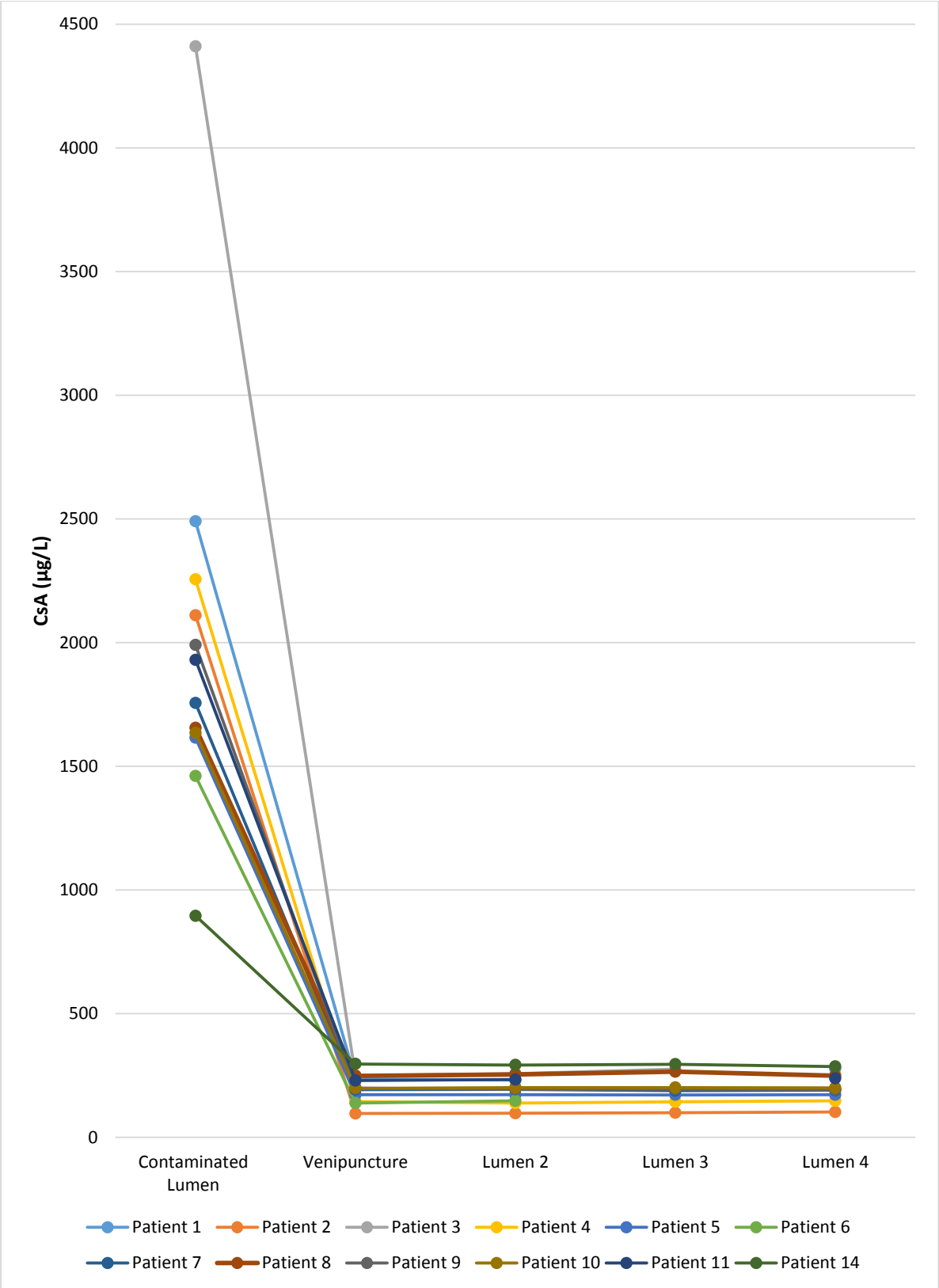

Figure 13. Data from patients 1-11 and 14, who received CsA.

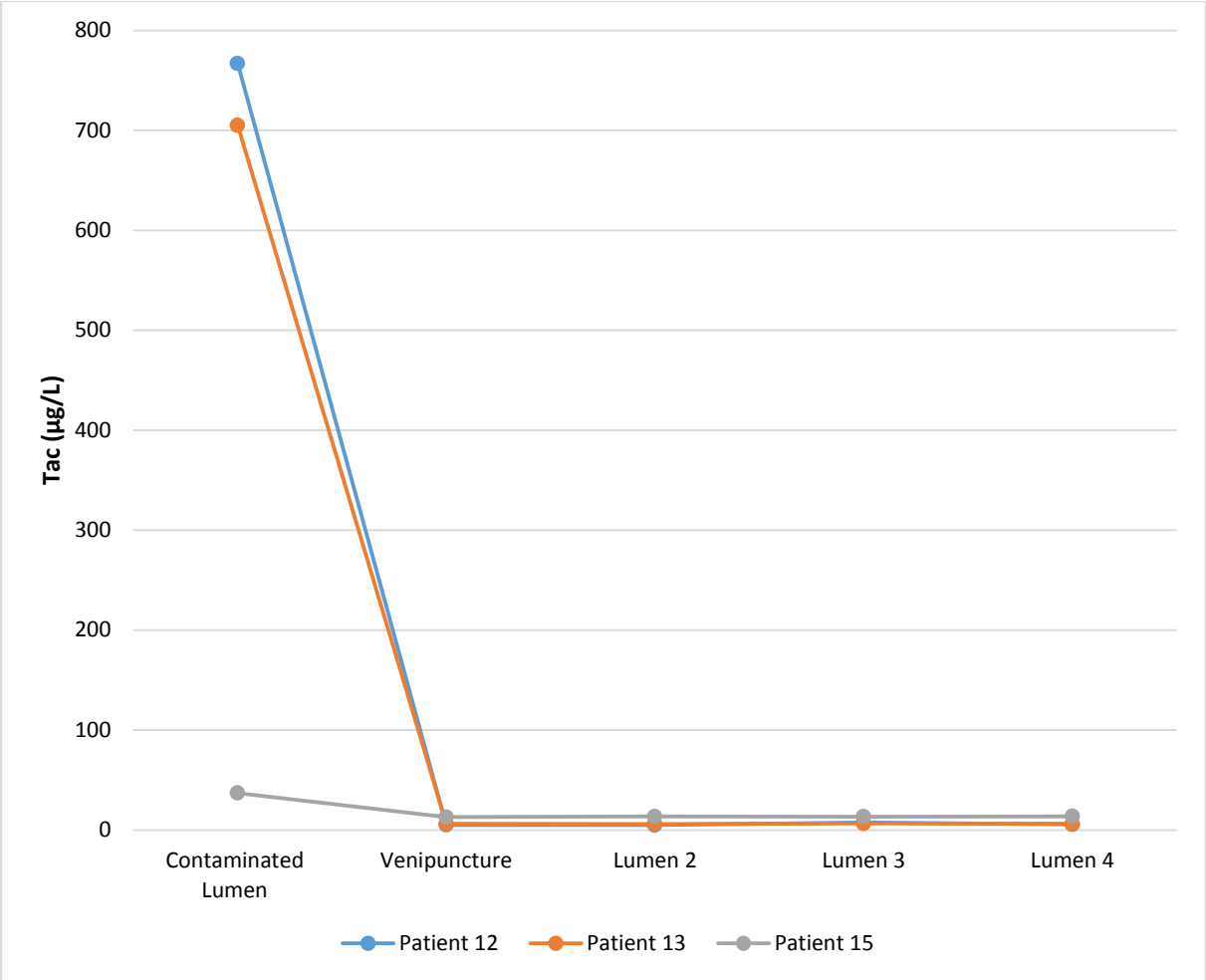

Figure 14. Data from patients 12, 13 and 15, who received Tac.

5.2.2. Special Cases

Patient 14, who had been switched to oral medication three days prior to blood sampling, still showed an increase of CsA concentration from the contaminated lumen (895 µg/L) by the factor of 3.0 compared to venipuncture (296 µg/L; cf. table 8) (Hacker et al. 2014).

In patient 15, the concentration from the lumen used only once for infusing Tac was elevated by a factor of 2.9. The sample received by venipuncture came to 12.9 µg/L and the one from the contaminated lumen resulted in 37 µg/L (Hacker et al. 2014).

Patient A was administered Tac through a CVC (Arrow-Howes™ PU CVC with three lumina) for 26 days. Sometime between day 22 and 25, the patient had most likely received an accidental overdose of Tac resulting in toxic concentrations of up to 111 µg/L measured on day 26. Tac medication was thus stopped completely, restarting with oral Tac five days after the withdrawal. Two days later, another toxic concentration of 86 µg/L of Tac was determined. However, it turned out that this time the sample had accidentally been taken from the lumen previously used for infusing Tac, since a control sample obtained via venipuncture yielded only 2.9 µg/L. Two more blood withdrawals taken 8 and 18 days after the last i.v. administration showed elevated blood concentrations in the contaminated lumen both by a factor of 37 compared to venipuncture (Hacker et al. 2014).

Similar to patient 12, the samples for patient A taken from the lumina previously not used for Tac administration did not fall within the range of ± 10 % of the values determined by venipuncture (cf. table 8) (Hacker et al. 2014).

6. Discussion

6.1. Comparison with Literature

The results from this systematic and comprehensive in vitro and in vivo study (cf. 5.1. and 5.2.) are in concordance with various reported cases (Blifeld and Ettenger 1987; Claviez et al. 2002; Grouzmann et al. 2008; Leson et al. 1989; Lorenz et al. 1991; Shaefer et al. 1993) as well as smaller in vivo (Busca et al. 1994; de Witte et al. 1986; Donnelly et al. 2003; Duffner et al. 1998; Jain et al. 1995; Lotfi et al. 2005; Senner et al. 2005) and in vitro (Carreras et al. 1988; Fitzsimmons and Ponzillo 1988) experiments for CsA. In all of the published articles, CsA or Tac are shown to reversibly adsorb to CVCs resulting in spuriously elevated concentrations if the blood sample is taken from the same lumen which had previously been used to administer the drug (Hacker et al. 2014).

In this study, for the first time, the development of reversible CsA and Tac adsorption was analyzed over the course of extensive rinsing experiments in a broad and systematic approach. Previously, only smaller in vitro experiments were reported which were limited to CsA (Carreras et al. 1988; Fitzsimmons and Ponzillo 1988). Moreover, CVCs with an incorporated silver ion-based antimicrobial agent had never been tested before. They were used in the in vitro experiments and a patient with a silver-Vy-CVC (cf. patient 15) was included in the in vivo study. This is the first reported case of adsorption and release of Tac to a silver-CVC in the literature (Hacker et al. 2014).

6.1.1. Recommendations on Blood Sampling for CsA and Tac Monitoring

Opinions in the literature are very heterogeneous concerning blood sampling for CsA and Tac monitoring.

A number of authors hold the view that it is safe to obtain blood samples from the same CVC previously used for infusing the immunosuppressant as long as an uncontaminated lumen is used for blood withdrawal:

Carreras et al. (Carreras et al. 1988) based this decision on their findings from six patients which had received CsA through a CVC. They did not find any significant difference between samples taken from the uncontaminated lumen of the CVC and samples taken from a peripheral vein ($n = 6$; $p = 0.16$; student's t-test). Unfortunately, the authors did not mention the material from which the CVCs were made.

Eight patients with CVCs made of silicone were administered CsA intravenously in a study conducted by Busca et al. (Busca et al. 1994). Since they did not observe any difference between samples obtained from the uncontaminated lumen of the CVC and via peripheral venipuncture, it was concluded that samples for CsA monitoring may be taken from either one of the two sites.

Senner et al. studied 71 paired CsA blood samples taken peripherally and from the CsA-uncontaminated lumen of double-lumen silastic CVCs (Senner et al. 2005). They did not find a significant difference between the two sampling sites (paired student`s t-test; $p = 0.13$) and concluded that blood sampling for CsA monitoring can be performed from the uncontaminated lumen of the CVC as long as the CsA infusion is not continuous.

After analyzing 49 paired samples from 10 patients with CVCs made of PU, Donelly et al. suggest that blood drawn from the uncontaminated lumen of a CVC can be used to monitor CsA levels after discarding 10 mL (Donnelly et al. 2003). The samples in their study were taken from the lumen of the CVC previously not used to infuse CsA as well as via

venipuncture. Still, they claim that this practice should not be conducted if the CVC is made of silicone due to stronger binding of CsA to this material.

Busca et al. conclude that there is no cross contamination in their 8 patients with silicone CVCs who had received CsA (Busca et al. 1994). Thus, blood sampling from the uncontaminated lumen of the CVC or from a peripheral vein would be adequate for CsA monitoring. Yet, their data shows that the lumen previously not used for infusing CsA (second lumen) indeed is contaminated in 11 of 19 cases. The CsA concentration from the samples taken from the second lumen of the CVC are 1.1 – 2.4 times higher (mean = 1.4) than the CsA levels from specimen taken via venipuncture. This is strong evidence against taking blood from a CVC when CsA monitoring is to be performed. This slight spurious elevation described in the cases of Busca et al. is exactly the kind of dangerous elevation which could remain undetected and lead to false dose adjustments (cf. 6.5.).

To avoid false dose modifications caused by spuriously elevated CsA concentrations from a contaminated CVC, de Witte et al. suggest that dose adjustments should be based on clinical side-effects of CsA as well as CsA concentrations taken from peripheral veins (de Witte et al. 1986).

All three articles in which Tac was used as an immunosuppressant come to the conclusion that it is not safe to withdraw blood for Tac monitoring from CVCs even if a different lumen was used to infuse the drug:

Even though no cross contamination was observed in their patients, both Shaefer at al. as well as Grouzmann et al. recommend venipuncture for therapeutic Tac monitoring (Grouzmann et al. 2008; Shaefer et al. 1993).

Jain et al. performed a broad study in 10 patients postulating that there are no significant differences in the concentrations of Tac in blood taken from an arterial line, via venipuncture or capillary blood (Jain et al. 1995). They then evaluated the CVCs which had been used for Tac administration. It was found that in seven of 13 samples the concentration from a lumen of the CVC previously not used for infusing Tac was 1.7 – 4.5 times higher than capillary /

arterial control blood samples. The samples obtained from the contaminated lumen were 3 – 23 times higher than capillary / arterial blood samples. Therefore, Jain et al. advocate capillary or peripheral venous blood sampling for the measurement of Tac concentrations since samples taken from the CVC are not reliable.

The results of our study (cf. 5.1. and 5.2.) confirm that blood sampling for TDM of CsA or Tac is only safe if obtained via venipuncture. Cross contamination did occur in three cases for Tac (cf. patients 12 and A), resulting in concentrations elevated by the factors of 1.3 (patient A), 2.4 (patient A) and 1.5 (patient 12). Considering these dangerous moderate elevations (cf. 4.), it is absolutely mandatory to take blood samples from peripheral sites if Tac is to be monitored. Taking blood from the CVC previously ever used to infuse CsA results in falsely elevated concentrations even after rinsing with high volumes of other fluids and discarding a limited volume of blood prior to blood sampling (cf. 5.1.1., 5.1.5. and 5.2.). Thus, we recommend for the sake of the patient, that blood sampling for TDM of CNIs should always be performed via venipuncture. Even if this means frequent hurtful procedures for the patient, this clearly is the only way to obtain safe and reliable CsA and Tac concentrations which can guarantee the best care for the patient.

6.1.2. Different CVC Materials

The only in vitro experiments comparing different CVC materials were conducted by Carreras et al. (Carreras et al. 1988). They put CVCs in a solution with CsA for 18h and measured the supernant CsA concentration afterwards. Since the supernant CsA concentration was lower than the initial concentration, they concluded that CsA had been adsorbed to the CVCs. The decrease of concentration was higher for silastic (50 %) and silicone (39 %) than PU (13.7 %). This falls in line with the in vitro experiments described above (cf. 5.1.2.). Here, it was found as well that CVCs made of silicone adsorb and release CNIs more than CVCs made of PU or silver. For the first time, our study reveals that this is not only the case for CsA as described by Carreras et al. but also for Tac.

6.1.3. Rinsing Aspects

Busca et al. report one case in which the CsA concentrations, taken from the lumen of a silicone CVC previously used for infusing the drug, were not elevated (Busca et al. 1994). The CsA infusion had been discontinued for 60h when a sample taken from the contaminated lumen yielded a concentration of CsA equivalent to those from samples drawn via venipuncture.

A similar scenario is presented by Leson et al. (Leson et al. 1989). Four patients with single-lumen CVCs made of silicone had received CsA through the CVC. CsA concentrations, taken from the CVC on day 8, 8, 10 and 11, respectively, after switching to oral CsA administration, were not elevated anymore. A possible reason could be the high amount of fluids (inter alia: total parental nutrition, sodium containing amino acids and dextrose, 10 % fat emulsion, standard dextrose, NaCl, antibiotics) administered through the single lumen after discontinuation of the CsA infusions. They could have washed CsA out of the lumen.

A small in vitro study reported by Fitzsimmons and Ponzillo affirms this theory (Fitzsimmons and Ponzillo 1988). It shows that flushing the CVC with 1 L of 5 % dextrose in water lowers the released CsA concentration but still results in remarkable drug release of 75 µg/L and 52 µg/L compared to > 600 µg/L before flushing with dextrose water.

Flushing of the CVC could also be an explanation in the case reported by Busca et al..

The reports by Busca et al. and Leson et al. stand in contrast with the in vivo study presented here (cf. 5.2.) where the concentrations from a contaminated lumen were always higher than the control sample obtained by venipuncture even if the patient had already been switched to oral CsA or Tac administration. Still, the in vitro study reveals (cf. 5.1.) that rinsing the catheter with fluids does lower the amount of CsA and Tac released from the CVC. Therefore, CsA and Tac cannot be detected anymore as long as the CVC is rinsed with high enough volumes of fluids, even if the required volumes might be very high (cf. 5.1.1.). Critically ill patients such as the ones who just underwent stem cell transplantation usually need high amounts of fluids over a long period of time. This makes it possible that CsA and

Tac which has adsorbed to the CVC might be washed out completely at some point if the drug was switched to oral administration and the CVC is still used regularly for other fluids. To further investigate this phenomenon, more in vivo studies should be conducted regarding the influence of rinsing on CNI release from CVCs.

6.1.4. Switch to Oral CsA Administration

It was shown in 11 patients by Lotfi et al. that if CsA is only administered orally no elevated concentrations are found in blood samples taken from the CVC (Lotfi et al. 2005).

Claviez et al. released a case report on one patient who had been administered CsA via a double lumen silicone CVC for 24 days (Claviez et al. 2002). 34 days after switching to oral CsA medication, CsA levels from the lumen not used to administer CsA were higher than the ones obtained via venipuncture (203 µg/L vs. 55 µg/L). As expected, the concentration from the lumen previously used for CsA infusion was elevated as well. Even 92 days after the switch to oral CsA, concentrations taken from the contaminated lumen of the CVC were still raised. However, 157 days after the first oral CsA dose, CsA concentrations from the formerly contaminated lumen were equivalent to the ones obtained via venipuncture. The authors suggest that diffusion of absorbed CsA from the silastic material into the catheter lumen might be the reason for the observation.

This case adds to the findings of the above described in vitro and in vivo study (cf. 5.2.). Patients 14, 15 and A also show elevated CNI concentrations in the contaminated lumen after switching to oral drug administration. However, patient A had received Tac orally for the last 18 days which was the longest time elapsed in this study. It is interesting to see that even after 92 days (Claviez et al. 2002), CsA still is released from a CVC. This underlines how much physicians have to be aware of this phenomenon at all times, even if CsA or Tac have not been infused for a long period of time.

6.1.5. Correction Factor

Analyzing four patients who were receiving CsA via CVCs made of silicone led Leson et al. to conclude that a correction factor for the CsA elevation in the contaminated lumen could be used (Leson et al. 1989). In contrast to their data, both the above described in vitro as well as in vivo experiments (cf. 5.1. and 5.2.) show that the adsorption and release of the drugs are of varying degree. Thus, they cannot be predicted precisely. This suggests that there is no fix correlation between the extent of the drug's elevation versus the dosage as well as the duration of the drug application (Hacker et al. 2014). However, in two out of three blood samples taken from patient A (cf. 5.2.2. and table 8), the Tac concentration taken from the contaminated lumen was 37 times higher than the one obtained via venipuncture. To further investigate this interesting correlation, further studies should be conducted with a larger number of cases.

6.2. Technical Aspects of the in Vitro Study

Conducting the mimicked blood sampling with whole EDTA blood showed similar results as compared to FFP (cf. figure 11 and 12). Moreover, performing the experiments in a water bath at 37° C only raised the drug concentration to a small degree (cf. 5.1.3.). This verifies that the setup of the main in vitro experiments was close to in vivo conditions (Hacker et al. 2014). Even if the values obtained in the in vitro studies were slightly higher than they would be expected in vivo, this could mean that physicians are even more at risk of underdiagnosing spuriously elevated CsA or Tac concentrations since the false elevation might be of small degree (cf. 4.).

Mimicking blood sampling in vitro was performed retrogradely (in the sense of infusing something in vivo). This was done due to technical reasons of the in vitro setup. By infusing FFP retrogradely with a syringe, it was made sure that uncontaminated FFP was infused into the CVC and the contaminated portion could easily be collected into small Eppendorf tubes at the exit of the CVC (cf. 11.2., image 6). To ensure that performing the experiments

retrogradely did not falsify the concentrations of CsA and Tac, a set of special experiments was performed (cf. 4.1.6.). The results confirm that the direction of sampling FFP had no significant effect on the released drug concentrations (cf. 5.1.6.).

A unique characteristic of this in vitro study is that it compared different materials of CVCs (cf. 6.1.2.). One striking finding reveals that silicone-Vy-CVCs behave differently compared to the other types of CVCs used in the experiments (PU-AH-CVCs, PU-In-CVCs and silver-Vy-CVCs): Silicone-Vy-CVCs adsorb and release more CsA and Tac than the other CVCs (cf. 5.1.2.). This might be due to material properties of silicone which might allow for different interactions between the material and the drugs. To deepen these findings, further studies should be conducted. Moreover, the values of the released drug concentrations increase from P1 to P5 when five consecutive samples are taken (cf. 5.1.2.). This is a distinct characteristic of silicone-Vy-CVCs. In contrast, concentrations from P1 to P5 decline in all of the other CVCs. Similarly, the CsA and Tac levels decrease in the cases of silicone-Vy-CVCs from P5 to the following P1 while they augment in CVCs made of all other materials (cf. 5.1.2.). Even though these phenomena are very striking, their reason remains currently unknown. Since these kinds of experiments have never been reported in the literature previously, there clearly is the need to confirm these findings by performing further experiments.

The aspect that CsA as well as Tac concentrations increase from P5 to the following P1 in the cases of CVCs made of PU and silver came as a surprise (cf. 5.1.2.). It would have been expected that all released immunosuppressant levels slowly decrease as the CVCs are being rinsed with increasing volumes of fluids. But the detailed in vitro experiments show that the amount of the released drug can never be predicted precisely. One possible reason for the increase from P5 to P1 is the fact that the CVCs were rinsed with NaCl between the two measurements. On the one hand this could result in chemical reactions between the drug, the CVC material and NaCl, leading to a stronger drug release after the rinsing. On the other hand, there was quite a time delay between taking P5 from the previous set of experiments to P1 which was taken after the rinsing in the consecutive set of experiments. This period of

time might have also caused more molecules to be prone to release at the next mimicked blood sampling with FFP. Unfortunately, there can only be speculations about the reasons for the above mentioned phenomenon.

Throughout the in vitro experiments, the adsorption and release effect tended to be stronger for Tac than CsA. This assumption is based on several aspects: The maximum rinsing volume of NaCl which still resulted in detectable drug concentrations was higher if the CVCs had been infused with Tac compared to CsA (cf. 5.1.1.). This was true for all types of materials. It was possible to rinse the CVCs with about double the volume of NaCl and still detect Tac being released in contrast to CsA. Moreover, the experiments with fat emulsion also suggest that Tac could be detected for a longer period of time if the CVC was continuously rinsed with fat emulsion as well as NaCl (cf. 5.1.5.). In the case of the CVC which had been infused with CsA, 1.02 L of NaCl as well as 100 mL of fat emulsion could be used to rinse the CVC. More rinsing fluids resulted in undetectable CsA levels. The CVC infused with Tac, on the other hand, still showed measurable Tac release after being rinsed with 2.02 L of NaCl and 150 mL of fat emulsion.

6.3. Guidelines on CVC Use

Many guidelines on CVC use lack information about blood sampling for therapeutic drug monitoring. They do not call attention to the adsorption and release of CNIs or possible other drugs and substances (Bishop et al. 2007; Johns Hopkins Medicine. Interdisciplinary Clinical Practice Manual. 2015; Loveday et al. 2014; Moureau 2004). However, some guidelines are well aware of this phenomenon and recommend blood sampling for therapeutic drug monitoring via venipuncture or a native lumen if the drug has previously been infused through the same device (Great Ormond Street Hospital. Clinical guidelines. 2015; National Guideline Clearinghouse 2015).

The package inserts for CsA and Tac only mention PVC incompatibility with potential extraction of phthalates regarding drug preparation for infusion but not in connection with

blood sampling for therapeutic drug monitoring (Astellas Prograf U.S. Physician Prescribing Information. 2015; Fachinformation Prograf 5 Mg/Ml Konzentrat Astellas. ; Fachinformation Sandimmun® 50 Mg/Ml ; Hacker et al. 2014; Transplants by Donor Type 2015).

It is critical that medical stuff performing blood sampling on patients with CVCs are aware of the adsorption and release phenomenon resulting in spuriously elevated CNI concentrations. In the future, all guidelines and practice manuals should contain this information to make sure that no patient is harmed.

6.4. Possible Complications for Patients with TTP/HUS

A rare but very severe complication after hematopoietic stem cell or solid organ transplantation is TTP/HUS which can be induced by CsA or Tac (cf. 2.1.1.) (Dlott et al. 2004). The most important treatment option is thus discontinuation or sometimes dose reduction of the CNIs (Agarwal et al. 1995; Ho et al. 2005). The above described study suggests that the adsorption of CNIs to CVCs is not only reversible when blood is withdrawn but also when fluids are infused through the contaminated lumen (cf. 5.1.). This could possibly lead to small amounts of the CNIs still being washed into the body with other fluids long after the drug administration was discontinued. If 1 L of fluid was to be infused through the contaminated lumen of the patient and assuming that the drug was released with the same mean concentration measured in vitro after discarding only 2 mL of FFP (cf. 5.1.1.), a total amount of 6.42 mg of CsA or 0.25 mg of Tac could unintentionally be infused into the patient's body. Based on a study conducted by Shibata et al., it is also known that CsA and Tac adsorb to infusion sets made of PVC (Shibata et al. 2000). If further amounts of the drug are released from the infusion set, this could increase the extent of CNI accidentally infused into the patient. Depending on the patient's course of treatment, it should be left to the physician's discretion whether or not to tolerate these comparably small amounts of the drug. However, there clearly is a need to further investigate this possible phenomenon. If the patient is suffering from TTP/HUS and CNIs are to be withdrawn completely, the lumen previously

used for infusing CsA or Tac should not be utilized any longer or the CVC should be changed (Hacker et al. 2014).

6.5. Consequences of the Findings

Especially the first blood sample of the in vitro experiments yielded a multiple of the concentration of the reference range of CsA and Tac (i.e. 100-400 µg/L for CsA and 5-20 µg/L for Tac ; (UpToDate: Cyclosporine (systemic): Drug information 2015; UpToDate: Tacrolimus (systemic): Drug information 2015); cf. 5.1.1.). In the case of the in vivo study, samples taken from the contaminated lumen resulted in strikingly high drug levels as well (cf. table 8). Thus, if a blood sample is accidentally taken from the contaminated lumen of a CVC, doctors usually detect the falsely elevated levels right away as they differ significantly from what would be expected. This may lead the physicians to question the highly elevated drug level and blood sampling may be repeated correctly. Since the spuriously high concentration of CsA or Tac would be deemed unlikely, the doctors would not make any changes of treatment based on the incorrect blood concentration. Thus, no harm is done to the patient (Hacker et al. 2014).

This might be different in the following constellation: rinsing the contaminated lumen of the CVC with high quantities of fluids does not put an end to the drug release. Even after 24.01 L of NaCl, 4.4 µg/L of Tac was still released from a contaminated lumen of a silver-CVC in the in vitro study (cf. 5.1.1.). Infusing fat emulsion, as being done quite often in critically ill patients with CVCs, did not stop the drug release, either (cf. 5.1.5.). Moreover, patients 14, 15 and A reveal that even long time after switching to oral CsA or Tac administration, the lumen previously used for infusing the immunosuppressant remains contaminated (cf. table 8). Thus a blood sample taken from the lumen formerly used to administer CsA or Tac might result in only slightly elevated drug concentrations if the immunosuppressant had not been infused through the lumen for a while and the lumen had then been utilized to administer other fluids. In this dangerous constellation, the spurious elevation might be of a more

moderate degree. The CsA or Tac value might thus still be within or only moderately above the reference range. In contrast to the above mentioned case in which the concentration is alarmingly high resulting in further investigation, a moderately elevated concentration might not be noticed as being falsely elevated. As the physician might not be aware of the falsely elevated immunosuppressant concentrations, the phenomenon may falsify the patient's drug levels over an extended period of time. But even one spuriously raised concentration might lead to treatment adjustment resulting in erroneous dose reduction. This could put patients in undiscovered, dangerous underdosage since the falsely elevated CsA or Tac concentrations suggest that the patients' drug values are still within the desired range. Underdosage of immunosuppressive drugs would put patients after stem cell transplantation at risk for GVHD (Ram et al. 2012; Rogosheske et al. 2014) and patients after solid organ transplantation could even suffer from organ rejection (Geissler and Schlitt 2009) (Hacker et al. 2014).

This emphasizes that a lumen ever used for infusing CsA or Tac needs to be avoided for taking blood samples for TDM of CsA or Tac at all times. This holds true even if the patient has already been switched to oral medication or large quantities of other fluids have been administered through the contaminated lumen. Especially after a long time has passed since the last i.v. dose of CsA or Tac, it is absolutely critical to still be aware of this potentially very dangerous phenomenon (Hacker et al. 2014).

7. Summary

Cyclosporine A (CsA) and tacrolimus (Tac) have been cornerstones in immunosuppressive therapy for the last three decades. To guarantee optimal immunosuppression in patients, it is crucial that the concentration of the drug remains within narrow therapeutic ranges. Spuriously elevated concentrations of CsA and Tac can be caused by reversible adsorption of the drug to central venous catheter (CVC) systems. If blood samples for therapeutic drug monitoring are obtained from the same lumen of the CVC which was previously used for infusing the drug, CsA and Tac concentrations will be falsely and unpredictably increased. If undetected, this may lead to false dose reduction resulting in underdosage which may even entail organ rejection or graft-versus-host disease.

This thesis systematically examines this adsorption and release effect of CsA and Tac in vitro as well as in vivo. Four types of CVCs were examined in vitro: two made of polyurethane (PU), one from silicone and one from PU with an incorporated silver ion-based antimicrobial agent. In each experiment, one dose of CsA or Tac was infused into one lumen. After rinsing the catheter with increasing volumes of NaCl (0.9 %), blood sampling was mimicked with fresh frozen plasma (FFP) from the same lumen previously used for infusing the drug. In addition to the in vitro study, blood samples of fifteen patients were taken simultaneously from all lumina of the CVC and via venipuncture.

All CVCs analyzed in vitro showed significant reversible adsorption of CsA ($n = 13$; $p = 0.001$) and Tac ($n = 13$; $p = 0.001$, Wilcoxon signed rank test). Immediately after infusing the drugs, mean concentrations of 6420 µg/L of CsA ($n = 12$) and 250 µg/L of Tac ($n = 12$) were measured. Flushing the CVCs with NaCl or fat emulsion lowered the extent of the reversible adsorption but even high volumes still resulted in detectable drug release.

The samples obtained from the contaminated lumen in the in vivo study showed mean elevations by a factor of 11 for CsA ($n = 12$) and 89 for Tac ($n = 3$) compared to those taken via venipuncture. Cross contamination to neighboring lumina was observed in patients receiving Tac.

Therefore, it can be concluded that for the sake of the patients, blood sampling for immunosuppressant monitoring should never be performed from catheters previously used for infusing the drug even after prolonged periods of time and extensive rinsing.

8. Zusammenfassung

Seit drei Jahrzehnten sind Cyclosporin A (CsA) und Tacrolimus (Tac) die Grundpfeiler immunsuppressiver Therapie. Um eine optimale Immunsuppression von Patienten zu gewährleisten, ist es unabdingbar, dass die Konzentrationen dieser Medikamente innerhalb der engen therapeutischen Bereiche liegen. Falsch hohe Konzentrationswerte von CsA und Tac können durch reversible Adsorption der Medikamente an Zentralvenenkatheter (ZVKs) hervorgerufen werden. Wenn Blutproben zur Konzentrationsbestimmung aus demselben Schenkel des ZVKs entnommen werden, durch den zuvor das Medikament infundiert wurde, werden unvorhersehbar falsch hohe Werte erreicht. Sollte dies unbemerkt bleiben, so kann es zur Reduzierung der Dosis mit folgender Unterdosierung kommen, was sogar Organabstoßung oder Graft-versus-Host-Reaktion nach sich ziehen kann.

Die vorliegende Dissertation analysiert systematisch Adsorption und Freisetzung von CsA und Tac sowohl in vitro als auch in vivo. Vier Arten von ZVKs wurden in vitro untersucht: zwei aus Polyurethan (PU), einer aus Silikon und einer aus PU mit einer antimikrobiellen Silberbeschichtung. Bei jedem Experiment wurde eine Dosis CsA oder Tac in einen Schenkel eines ZVKs infundiert. Nach Spülung des Katheters mit steigenden Mengen an NaCl (0.9 %), wurden Blutabnahmen mit Fresh Frozen Plasma (FFP) aus dem Schenkel imitiert, durch den zuvor das Medikament infundiert wurde. Zusätzlich zu der In-vitro-Studie, wurde fünfzehn Patienten gleichzeitig Blut aus allen Schenkeln des ZVKs sowie durch periphere Venenpunktion entnommen.

Alle in vitro untersuchten ZVKs zeigten eine signifikante, reversible Adsorption von CsA ($n = 13$; $p = 0.001$) sowie Tac ($n = 13$; $p = 0.001$, Wilcoxon signed rank test). Unmittelbar nach der Infusion der Medikamente wurden durchschnittliche Konzentrationen von 6420 µg/L für CsA ($n = 12$) und 250 µg/L für Tac ($n = 12$) gemessen. Wurden die ZVKs mit NaCl oder Fettemulsionen gespült, so verringerte sich das Ausmaß der reversiblen Adsorption, doch auch große Mengen an Spülflüssigkeit führten immer noch zu nachweisbarer Freisetzung der Medikamente.

Die Proben, die bei der In-vivo-Studie aus dem kontaminierten Schenkel entnommen wurden, zeigten eine Erhöhung der Konzentration um das 11-fache bei CsA und das 89-fache bei Tac verglichen mit denen, die die durch periphere Venenpunktion entnommen wurden. Eine Kreuz-Kontamination der Nachbarschenkel wurde bei Patienten beobachtet, die Tac erhielten.

Deshalb lässt sich zusammenfassend sagen, dass zum Wohle des Patienten Blutentnahmen zur Spiegelbestimmung von Immunsuppressiva niemals aus den ZVKs erfolgen sollten, durch die zuvor das Medikament infundiert wurde. Dies gilt auch, wenn seitdem schon lange Zeit vergangen ist und ausgedehnte Spülungen erfolgt sind.

9. List of Tables and Figures

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11. Appendix

11.1. Publications

11.1.1. Posters

Abstract Award:

Hacker C, Steimer W. Falsely Elevated Immunosuppressant Concentrations: Reversible Adsorption to a Variety of Central Venous Catheters [Poster]. American Association for Clinical Chemistry Annual Meeting, 2011, July 24th-28th, Atlanta, USA.

Poster:

Hacker C, Menzel H, Steimer W. Spuriously high levels of immunosuppressants due to reversible adsorption to central venous catheters [Poster]. 12th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology, 2011, October 2nd-6th, Stuttgart, Germany.

11.1.2. Original Research Paper

Hacker C, Verbeek M, Schneider H, Steimer W. Falsely elevated cyclosporin and tacrolimus concentrations over prolonged periods of time due to reversible adsorption to central venous catheters. Clin Chim Acta 2014; 433: 62-68

11.2. Images

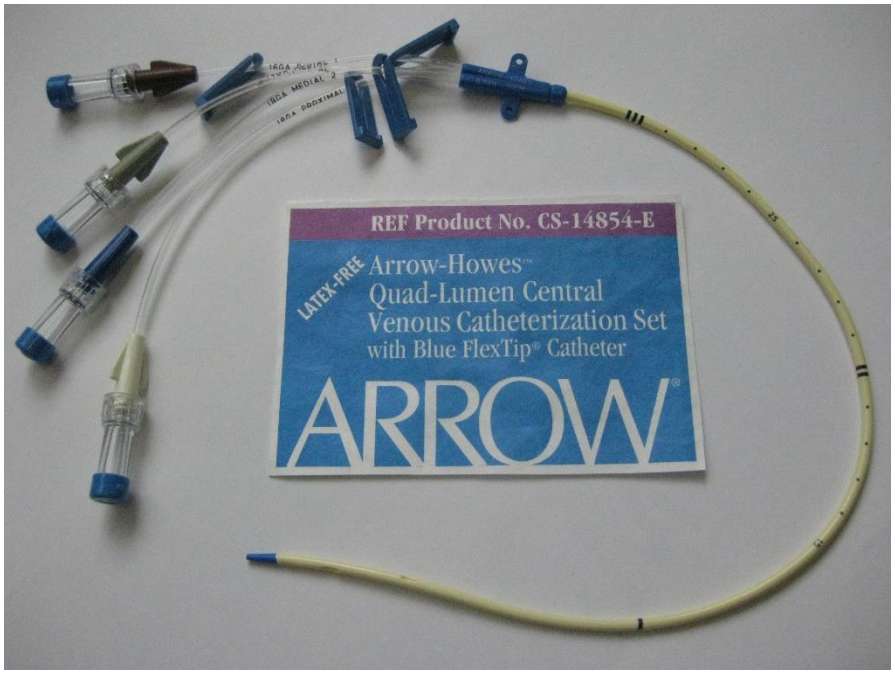

Image 1. Arrow-Howes™ "Quad-Lumen Central Venous Catheterization Set" (PU-AH-CVC)

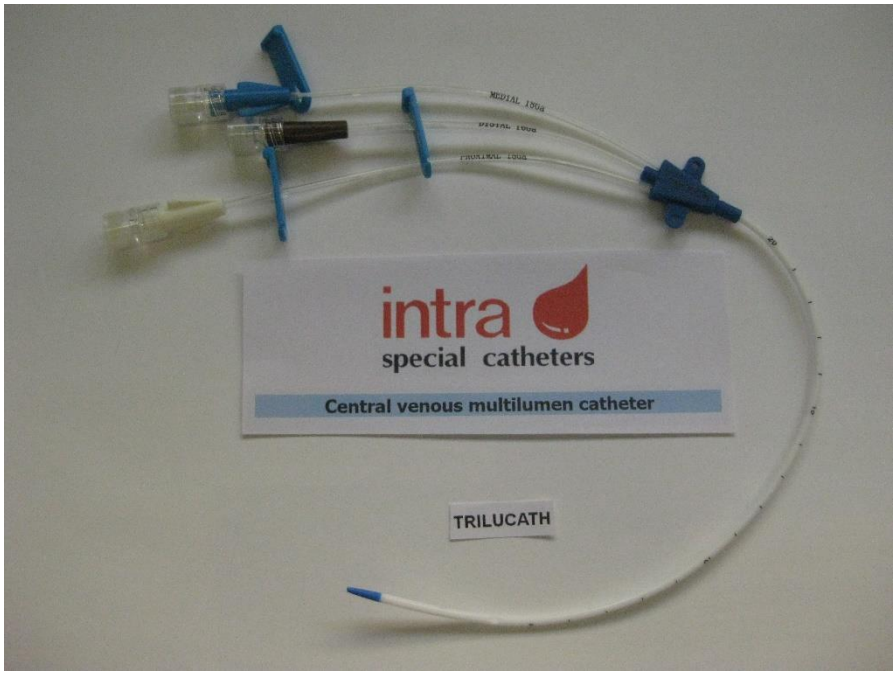

Image 2. Intra "Trilucath" (PU-In-CVC).

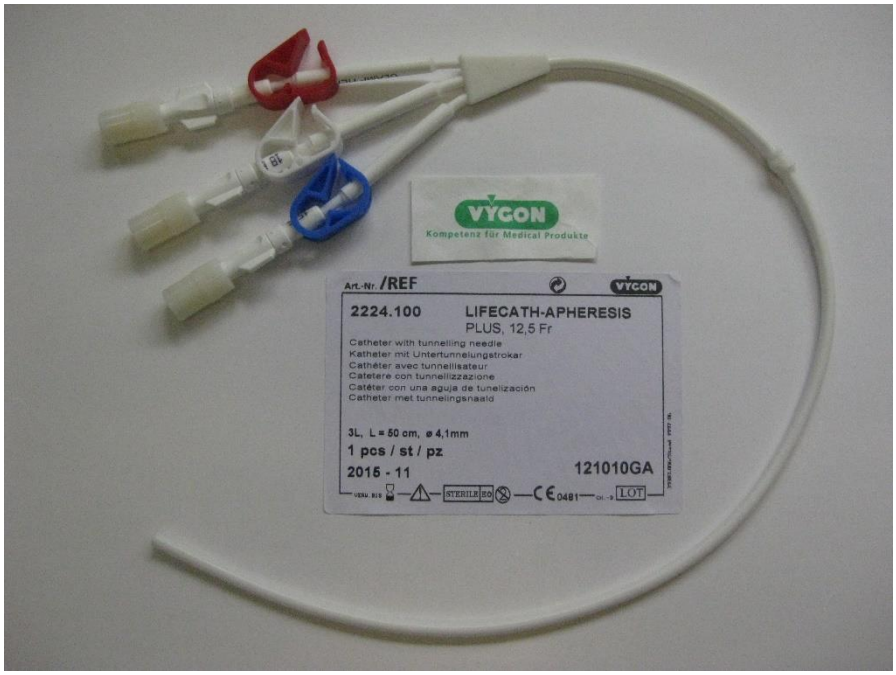

Image 3. Vygon "Lifecath Apheresis Plus" (silicone-Vy-CVC).

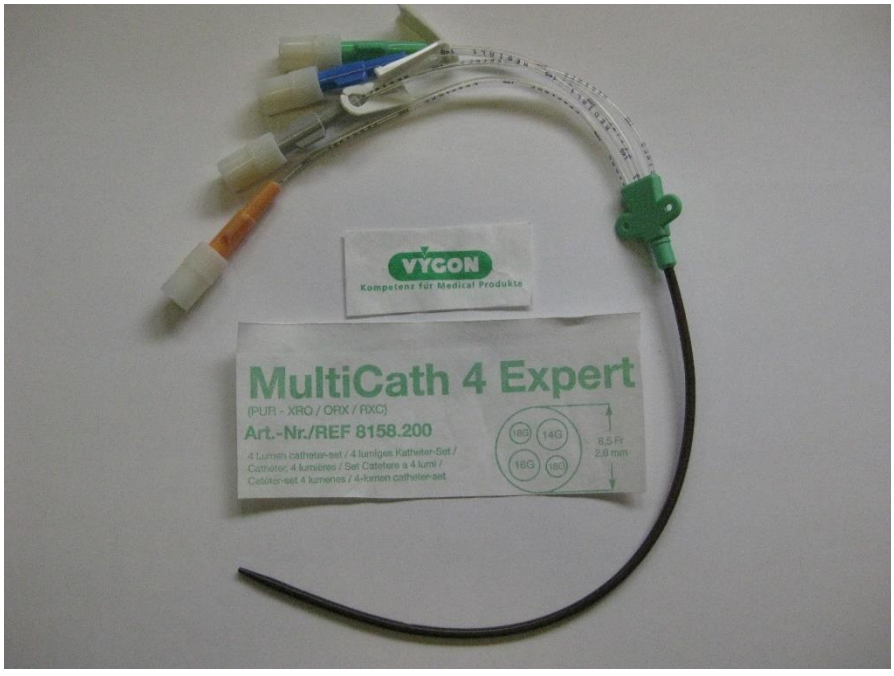

Image 4. Vygon "Multicath 4 Expert" (silver-Vy-CVC).

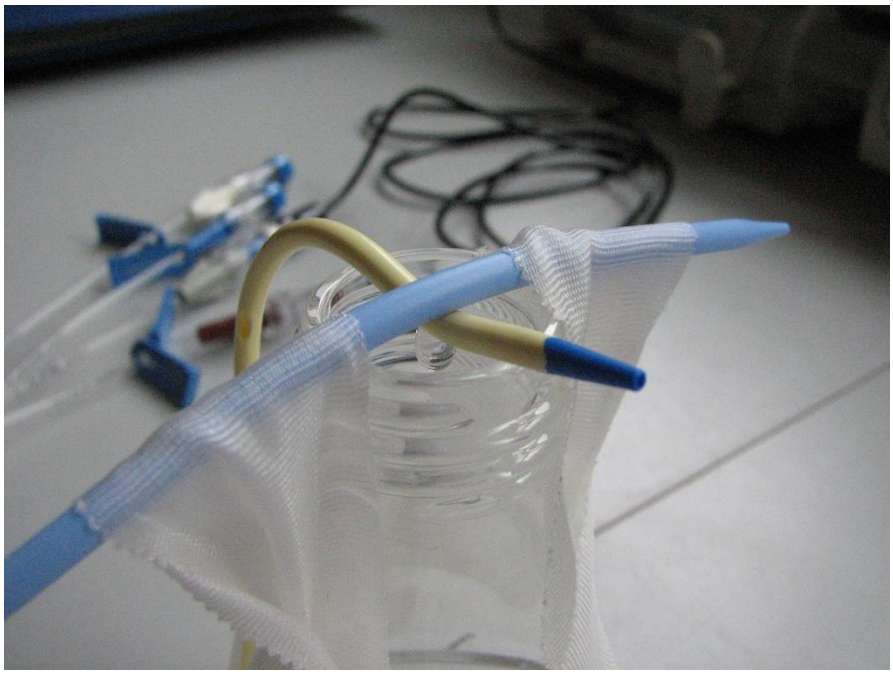

Image 5. Infusing CsA or Tac without cross contamination at the exit of the CVC.

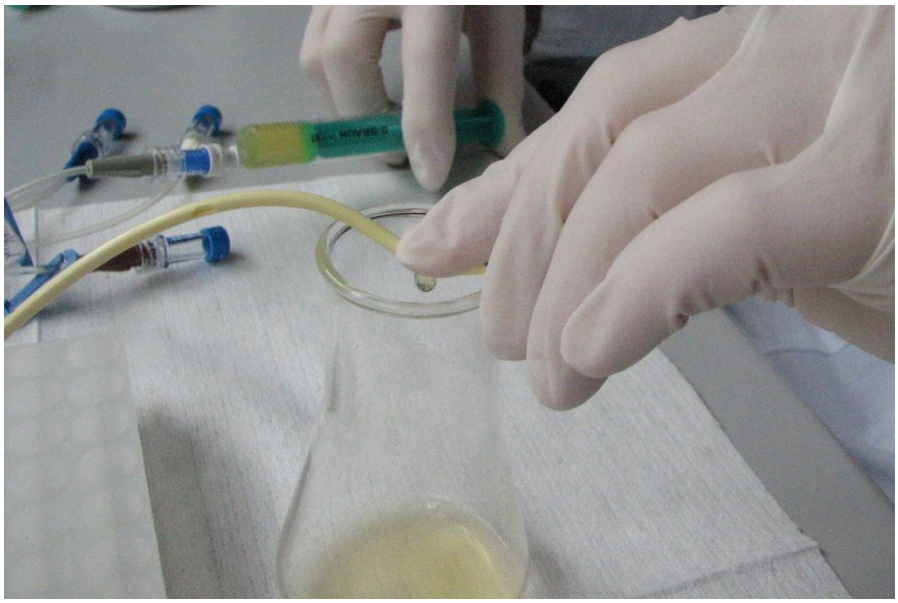

Image 6. Mimicking blood sampling: infusing FFP retrogradely with a syringe.

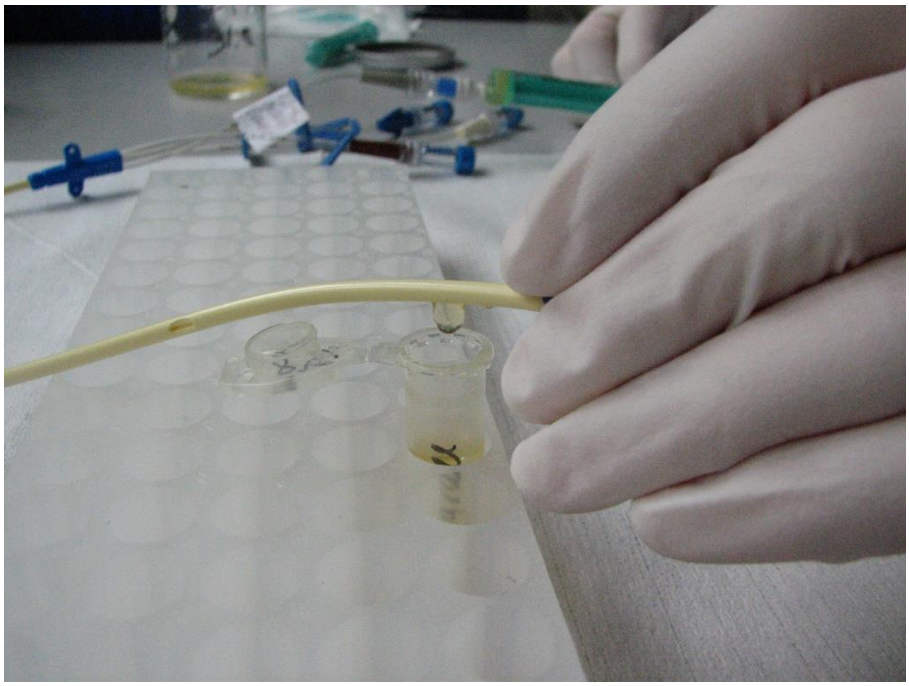

Image 7. Mimicking blood sampling: collecting portions of 2 mL into small Eppendorf tubes at the exit of the CVC.

PATIENTENAUFKLÄRUNG

Zur Studie „Arzneimittelspiegelbestimmung von Immunsuppressiva – entnommen aus Zentralvenenkathetern sowie per Venenpunktion“

Sehr geehrte Patientin, sehr geehrter Patient,

bei Ihnen soll im Rahmen einer Studie eine Blutentnahme durchgeführt werden. Wie Sie es bereits aus Routineuntersuchungen kennen, soll Ihnen diesmal aus jedem Schenkel Ihres Zentralvenenkatheters eine Blutprobe (je 2,7ml) abgenommen werden. Zudem soll noch eine weitere Blutprobe aus einer Vene entnommen werden. Dies geschieht fast immer in der Armbeuge.

In den Proben wird dann jeweils der Spiegel Ihres Immunsuppressivums bestimmt, also wie hoch die Konzentration des Medikamentes ist. Wir gehen nämlich davon aus, dass Immunsuppressiva an der Wand des Zentralvenenkatheters gleichsam „kleben“ bleiben. Wenn nun eine Blutentnahme aus demselben Schenkel erfolgt, durch den zuvor das Immunsuppressivum gegeben wurde, dann löst sich augenscheinlich ein Teil des Medikamentes wieder und wird in die Spritze gesaugt. So kann es zu einer zu hohen Konzentration des Medikamentes in der Blutprobe zukommen. Die auf diese Weise gewonnenen Werte, die im Labor gemessen werden, können in der Folge dann höher sein als die eigentliche Konzentration, die in Ihrem Körper herrscht. Um die Ergebnisse gegebenenfalls nochmals überprüfen zu können, werden die Blutproben für maximal 10 Jahre im Institut für Klinische Chemie und Pathobiochemie des Klinikums Rechts der Isar aufbewahrt.

Die einzige Belastung, die bei einer Teilnahme an der Studie für Sie auftritt, ist die einmalige Blutentnahme (insgesamt höchstens 13,5ml), die im Rahmen routinemäßig notwendiger Blutentnahmen durchgeführt werden kann. Zu den Risiken der Blutabnahme gehört das Entstehen blauer Flecken im Bereich der Einstichstelle. Es besteht das sehr geringe Risiko einer lokalen oder allgemeinen Infektion. In extrem seltenen Fällen kann es zu einer Verletzung eines Hautnervs, evtl. sogar mit chronischem Verlauf, kommen.

Die Teilnahme an der Studie erfolgt freiwillig und kann jederzeit ohne Angabe von Gründen und ohne Nachteile für Sie von Ihnen abgebrochen werden.

Alle persönlichen Daten sowie die Ergebnisse der Untersuchung unterliegen der ärztlichen Schweigepflicht. Sie werden nicht an Dritte weitergegeben. Die Ergebnisse der Studie werden pseudonymisiert, also ohne Angaben Ihres Namens, veröffentlicht.

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EINVERSTÄNDNISERKLÄRUNG

Zur Studie „Arzneimittelspiegelbestimmung von Immunsuppressiva – entnommen aus Zentralvenenkathetern sowie per Venenpunktion“

Ich erkläre mich hiermit mit dem Einschluss in die Studie einverstanden, insbesondere damit, dass mir einmalig Blut aus dem Zentralvenenkather sowie per Venenpunktion abgenommen wird. Dies geschieht ausschließlich zu dem Zweck, die Konzentration des Immunsuppressivums in den entnommenen Proben zu bestimmen. Ich stimme außerdem zu, dass ein Teil der Blutproben bis zu 10 Jahre im Institut für Klinische Chemie und Pathobiochemie des Klinikums Rechts der Isar aufbewahrt wird, um die entsprechenden Ergebnisse überprüfen zu können. Ich hatte Gelegenheit, alle Fragen zur Studie zu stellen. Mir ist bewusst, dass sich aus dem Ergebnis der Studie für mich keine Therapiekonsequenzen ergeben.

Alle persönlichen Daten sowie die Ergebnisse der Untersuchung unterliegen der ärztlichen Schweigepflicht. Sie werden nicht an Dritte weitergegeben. Die Ergebnisse der Studie werden pseudonymisiert, also ohne Angaben meines Namens, veröffentlicht.

Diese Einwilligungserklärung kann ich jederzeit widerrufen, ohne dass damit meine Behandlung in irgendeiner Weise beeinträchtigt wird.

Name, Vorname des Patienten: _____

München, den _____ _____

(Unterschrift des Patienten)

Name, Vorname des Arztes: _____

München, den _____ _____

(Unterschrift des Arztes)

12. Acknowledgements / Danksagungen

Zum Gelingen der vorliegenden Dissertationsarbeit haben zahlreiche Personen beigetragen, bei denen ich mich an dieser Stelle herzlich bedanken möchte.

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Meinem Doktorvater, Herrn Professor Steimer, möchte ich ausdrücklich für die Überlassung des Themas sowie die intensive Betreuung und wissenschaftliche Hilfestellungen über die Jahre danken. Sein stets offenes Ohr sowie seine Ratschläge haben mir die Arbeit sehr erleichtert.

Ohne den Einsatz und das Verständnis der Mitarbeiterinnen aus dem Drug Monitoring Labor wäre diese Arbeit nicht zustande gekommen. Hier möchte ich vor allem Frau Christine Müller und Frau Christine Völkl meinen tiefen Dank aussprechen. Zudem bedanke ich mich bei Frau Kerstin Siegmann und Frau Anna Büschges sowie allen weiteren Mitarbeiterinnen, die für die stets positive Arbeitsatmosphäre im Labor gesorgt haben.

Für die sehr gute Zusammenarbeit im Rahmen unserer Studie möchte ich der III. Medizinischen Klinik der TU München unter der Leitung von Herrn Professor Peschel danken. Herr Dr. Helge Menzel und Frau Dr. Mareike Verbeek haben sich häufig Zeit für meine Fragen auf der Stammzelltransplantationsstation genommen, wofür ich sehr dankbar bin. Auch die engagierten Pflegekräfte der Station 3/0 seien hier erwähnt.

Ferner danke ich Herrn Dipl.-Stat. Bernhard Haller sowie Herrn Dr. Alexander Hapfelmeier vom Institut für Medizinische Statistik und Epidemiologie der TU München für ihre statistische Beratung.

Meinen Eltern sowie meinem Bruder und allen voran Stephan Düwel gilt mein besonderer Dank für ihre kontinuierliche Unterstützung.