

**Limitations of current treatment strategies in human heart transplantation: Studies on pulmonary hemodynamics, renal failure and immunosuppression.**

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# 1. Acknowledgments

This thesis is based on clinical work and studies performed at the Department of Cardiology, Oslo University Hospital Rikshospitalet. The two first papers are based on our database established before I started working within the field of heart transplantation. I am thankful that I have been given the opportunity to use the database for my research and I am grateful to the initiators of the database and those contributed to keep it updated.

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## **2. Abbreviations.**

AKIN- acute kidney injury network  
ARF- acute renal failure  
AZA- azathioprine  
CAV- cardiac allograft vasculopathy  
CI- cardiac index  
CNI- calcineurin inhibitor  
CO- cardiac output  
CRF- cronic renal failure  
CsA- cyclosporine  
EVE- everolimus  
GFR- glomerular filtration rate  
HF- heart failure  
HTx- heart transplantation  
IL- interleukin  
ISHLT- International Society of Heart and Lung Transplantation  
LV- left ventricle  
MAP- mean arterial pulmonary pressure  
MMF- mycophenolatmofetil  
mTOR- mammalian target of rapamycin  
PCW- pulmonary capillary wedge pressure  
PH- pulmonary hypertension  
PSI- proliferation signal inhibitor  
PVR- pulmonary vascular resistance  
RAM- right atrial mean pressure  
RHC- right heart catheterization  
RHH- right heart hemodynamics  
RIFLE- risk, identification, failure, end stage renal failure  
SAP- systolic arterial pulmonary pressure  
TAC- tacrolimus  
TPG- transpulmonary gradient  
TTx- thoracic organ transplantation

### 3. List of Papers.

#### Paper 1:

**Pulmonary hypertension in heart transplantation: discrepant prognostic impact of preoperative compared to one year postoperative right heart hemodynamics.**

Einar Gude, Svein Simonsen, Odd R. Geiran, Arnt E. Fiane, Lars Gullestad, Satish Arora, Anne Relbo and Arne K. Andreassen.

J Heart Lung Transplant. 2010;29:216-223

#### Paper 2:

**Acute renal failure early after heart transplantation: risk factors and clinical consequences.**

Einar Gude, Arne K. Andreassen, Satish Arora, Lars Gullestad, Ingelin Grov, Anders Hartmann, Torbjørn Leivestad, Arnt E. Fiane, Odd R. Geiran, Mari Vardal, and Svein Simonsen.

Clin Transplant. 2010;24:207-213

#### Paper 3:

**Benefit of early conversion from CNI-based to everolimus-based immunosuppression in heart transplantation.**

Einar Gude, Lars Gullestad, Satish Arora, Svein Simonsen, Ina Hoel, Anders Hartmann, Hallvard Holdaas, Arnt E. Fiane, Odd R. Geiran and Arne K. Andreassen.

J Heart Lung Transplant. 2010;29:641-647

#### Paper 4:

**Improvement in renal function after everolimus introduction and calcineurin inhibitor reduction in maintenance thoracic transplant recipients: the significance of baseline GFR.**

Satish Arora\*, Einar Gude\*, Vilborg Sigurdardottir, Svend-Aage Mortensen, Hans Eiskjær, Gerdt Riise, Lena Mared, Øystein Bjørtuft, Bjørn Ekmehag, Kjell Jansson, Svein Simonsen, Pål Aukrust, Dag Solbu, Martin Iversen and Lars Gullestad.

J Heart Lung Transplant. 2012;31:259-265

\*both authors contributed equally to the present study

## **4. Introduction.**

### **4.1. Historical remarks.**

Heart Failure (HF) is an increasing health problem with an incidence of 0.5 % and a prevalence of 1-3 % in the western world. Ten percent of HF patients are in NYHA class IV with one year mortality up to 50 %<sup>1,2</sup>, depending on the response to modern HF treatment<sup>3,4</sup>. The treatment of choice for selected patients with the most dubious prognosis and the capacity to benefit, is heart transplantation (HTx)<sup>5,6</sup>.

The first human HTx was performed in South Africa by Dr. Bernard in 1967. With a 24 year old donor, the patient was improving hemodynamically, but succumbed 18 days after surgery due to pneumonia<sup>7</sup>. Steroids were deployed in clinical transplantation in the 1960s. Cyclosporine (CsA) was first used in renal transplantation in 1979 and introduced in HTx at Stanford University in California in 1980<sup>8</sup>. The diagnosis of rejection was improved by the introduction of Caves-Schulz biptome in 1975 and the Billingham rejection scoring system 1974. A more specific and balanced immunosuppression improved two years survival to nearly 80 %, an inspiration for the first Norwegian HTx in 1983<sup>9</sup>.

The first HTx patient in Norway is still alive and with excellent quality of life. Mean survival after HTx in Norway is  $12.3 \pm 5.3$  years, superior to most international results reported by the International Society of Heart and Lung Transplantation (ISHLT)<sup>10-12</sup>. Norway was in 2008 reported with 7.7 HTx/mill/year, with a maximum of 45 in 2003. However, due to donor organ shortage, only 30 to 35 HTx are performed in Norway yearly (fig 1), making each donor organ a special resource utilized to the best of potential recipients, a selection process following international guidelines.

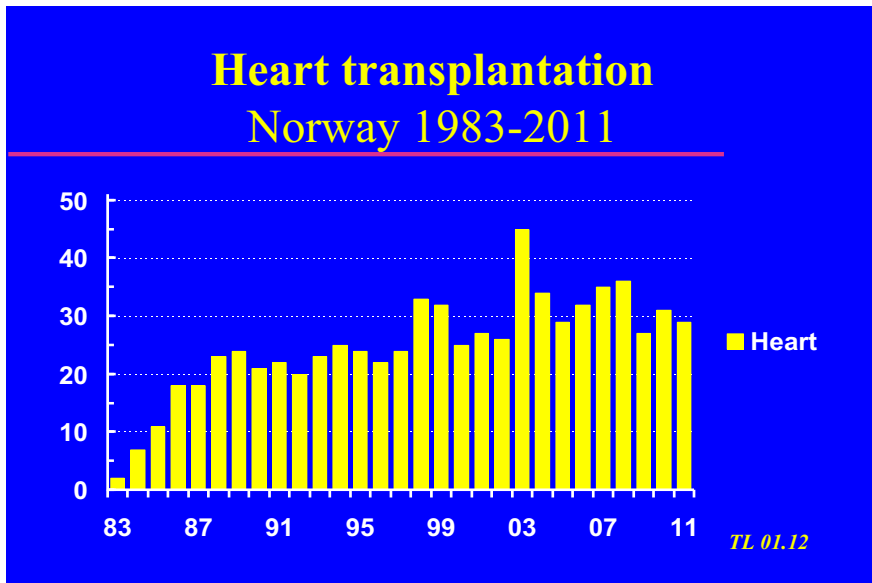


Figure 1. Number of Heart Transplantations performed yearly at Rikshospitalet. With courtesy to dr. T. Leivestad, Institute of Immunology. Oslo University Hospital Rikshospitalet.

#### 4.2. Hemodynamics before and after HTx.

HF involves important and crucial hemodynamic changes that require additional attention for optimal treatment as well as for risk stratification both pre- and post-HTx<sup>3;12;13</sup>. Pulmonary hypertension (PH) due to left sided heart failure is common and carries a poor prognosis<sup>14;15</sup>. Severe irreversible PH is one of the few absolute contraindications to HTx<sup>5</sup>. The rationale is that a normal donor heart is not capable of maintaining adequate right ventricular stroke volume against an elevated and fixed pulmonary vascular resistance (PVR)<sup>16;17</sup>. Just what constitutes unacceptably high levels of PVR, with or without the use of vasodilatation test, is still debated<sup>11;18</sup>. Guidelines concerning acceptable preoperative right heart hemodynamics (RHH) to prevent right heart failure in the implanted heart were initially developed at Stanford University. International guidelines are published by the ISHLT working group and



have been slightly liberalized compared to the original Stanford protocol<sup>12</sup>. The evidence for RHH guidelines is weak (class of evidence IIb) and is still based on small single centre reports. The value of repetitive right heart catheterization (RHC) while waiting for HTx is uncertain and the clinical and prognostic impact of elevated pulmonary pressures after HTx is scarcely described<sup>3;12</sup>.

### **4.3. Immunosuppression in HTx recipients.**

New immunosuppressive strategies have improved survival after HTx and many centers have based their immunosuppressive strategy on triple-drug regimen consisting of calcineurin inhibitors (CNI; CsA and tacrolimus (TAC)), azathioprine (AZA)/mycophenolatemofetil (MMF) and corticosteroids<sup>11;19</sup>.

CNI has contributed to lower rejection rates and favourable graft survival reaching 90 % the first year after HTx<sup>11</sup>. CsA is a prodrug and its immunosuppressive properties depend on the formation of a complex with cellular proteins called immunophilins consisting of the cyclophilin (which CsA binds to) and the FK binding system (which TAC binds to). The CsA- cyclophilin complex inhibits calcineurin, which is responsible for activating the transcription of interleukin 2 (IL-2) and other cytokines. Inhibition of IL-2 reduces activation and function of T-cells and blocks the T-cell cycle in the G0 phase and limits activation of cytotoxic T-cells<sup>7;20</sup>. CsA has been approved for use as a primary immunosuppressant for more than 35 years and advances in formulation design and therapeutic drug monitoring have resulted in substantial improvement in clinical outcome<sup>20-22</sup>.

TAC or FK-506 was discovered in 1984, launched in 1993, and has an increasing market among CNIs in HTx recipients, now reaching more than 50 %<sup>11;23</sup>. The mechanism of action and effect as an immunosuppressant agent are much similar to that of CsA<sup>22;24</sup>.

Prolonged TAC with dosing once daily is now available, possibly improving recipient compliance, but still not recommended in HTx guidelines<sup>22;25</sup>.

AZA, a purine analogue immunosuppressant was standard at our institution 1983-2002, and replaced by MMF, a reversible inhibitor of inosine monophosphate dehydrogenase in purine biosynthesis which is necessary for growth and replication of T and B cells. Introduction of MMF reduced rejection and showed superior survival compared to AZA<sup>20;23</sup>.

Corticosteroids are the third class of drugs to prevent rejection. Many centres have tried to withdraw steroids due to side effects<sup>5</sup>. In our centre, 90 % of the recipients use steroids.

Proliferation signal inhibitors (PSI), such as everolimus (EVE) and sirolimus, may represent an attractive renal sparing alternative to CNI therapy. They inhibit mammalian target of rapamycin (mTOR) causing blockage of T-cell activation and inhibits proliferation of smooth muscle cells by blocking cell replication in the G0 phase in addition to antiproliferative properties<sup>20;26</sup>. Multiple metabolites of the two agents make their immunologic properties uncertain<sup>19</sup>. Preliminary reports have demonstrated that these agents can replace CNI therapy in Tx recipients and allow an improvement in renal function without loss of immunosuppressant potency<sup>27-29</sup>. Others have reported high frequency of side effects of these agents<sup>30;31</sup>. Thus, there is still controversy about the degree of renal failure, to what time and whether as a substitute or in combination with CNI, that PSI should be introduced<sup>32</sup>. Compared to sirolimus, EVE has superior biochemical properties: higher bioavailability, shorter half time, faster absorption and no loading dose required<sup>33</sup>.

#### **4.4. Adverse effects of immunosuppression in HTx recipients.**

Although CNI inhibitors are first choice as main immunosuppressant in most centres, side effects include renal failure, hemodynamic perturbations and involvement in development of cancer and coronary allograft vasculopathy (CAV)<sup>11;34</sup>. Nearly 30 % of HTx recipients develop renal dysfunction as early as one year post-HTx, an independent risk factor for both all-cause and cardiac mortality<sup>35-37</sup>.

Acute renal failure (ARF) is a major contributor to morbidity and mortality in HF and cardiac surgery<sup>38;39</sup>. However, in the HTx setting the consequences of ARF are less well studied and most reports focus on long-term development of chronic renal failure (CRF)<sup>37</sup>. CNI induced nephrotoxicity occurs often within the first doses post-HTx due vasoconstriction of the preglomerular arterioles<sup>23</sup>. Chronic use of CNIs is characterized by glomerular sclerosis, interstitial fibrosis and thickening of capillary basement membranes seen in 25 % already six months post-HTx<sup>40</sup>. High prolonged CsA blood concentrations are known to predispose to chronic CNI induced nephrotoxicity<sup>19;20;41;42</sup>. Chronic renal damage due to a diverse spectrum of diagnosis is usually not responsive to dose reduction and therapeutic action must therefore be taken before fibrosis has been established<sup>32;42;43</sup>.

## **5. Main aims of the study.**

1. To evaluate indices of pulmonary hypertension pre- and post-HTx and their prognostic significance on survival.
2. To evaluate incidence and risk factors of ARF after HTx and the impact of ARF on prognosis.
3. To evaluate early and late conversion from CNI based to everolimus based immunosuppression in HTx with focus on safety and renal improvement.
4. To evaluate everolimus introduction and CNI reduction in maintenance TTx with emphasis on baseline GFR as marker of renal improvement.

## **6. Material and Methods.**

### **6.1. Study population.**

Oslo University Hospital Rikshospitalet is the only transplantation center in Norway and all HTx activity is located here. Patients were identified from our HTx database created in 1996. Data were retrospectively collected from 1983-96 and prospectively collected from 1996. All patients transplanted between 1983 and 2007 were included in studies on PH (n=500) and ARF (n=585). In paper 1, 53 recipients were not included due to age below 16 years, recipients of multiple organs, re-HTx, mechanical tricuspid or pulmonary valve and missing data.

In paper 3, 31 HTx recipients with severe renal failure defined as  $\text{eGFR} \leq 30 \text{ ml/min/1.73m}^2$  were included and divided in two groups according to time since HTx. Group 1 consisted of 16 patients transplanted within the last year [median 5.5 (1.3-8.5) months] before CsA elimination. One patient was not analyzed for primary end point due to less than 24 months follow up. Fifteen long term recipients were recruited (Group 2). Due to early withdrawal and deaths, 10 patients 81 (39-109) months since HTx were analyzed for change in renal function defined as primary end point.

Paper 4 was a 12-month, open-label, multicenter, randomized, controlled study in Scandinavia (inclusion period December 2005 to March 2008) where 282 maintenance thoracic organ recipients (TTx) (190 HTx and 92 lung Tx) were randomized to continue their current immunosuppressive regimen or start everolimus therapy with a pre-defined reduction in CNI exposure. In this thesis discussion will mainly focus HTx recipients.

## 6.2. Right heart catheterization.

RHC was performed using a Swan-Ganz pulmonary artery thermodilution catheter (Baxter Health Care Corp., Santa Anna, Ca., USA) or a Coumand catheter (for Fick method) before HTx, 2-3 weeks, 6 months and thereafter yearly for the first 3 years after HTx. RHC was repeated every third month while on waiting list for HTx.

The following pressures were recorded in mmHg: Right atrial mean pressure (RAM), systolic pulmonary artery pressure (SAP), mean pulmonary artery pressure (MAP) and mean pulmonary capillary wedge pressure (PCW). Cardiac output (CO) was measured by the Fick technique or thermodilution, and cardiac index (CI) calculated by dividing CO by body surface area. The transpulmonary gradient (TPG) was obtained by subtracting PCW from MAP and PVR (in Wood units (WU)) by dividing TPG by CO. For examination of CO, the Fick technique was used to measure CO the first 7 years, later replaced by the thermodilution technique<sup>44</sup>.

For listing of patients for HTx, two of the following three criteria had to be fulfilled:  $SAP \leq 50$  mmHg,  $TPG \leq 10$  mmHg and  $PVR \leq 2.5$  WU. If not, sodium-nitroprusside, for reversibility testing, was given intravenously (iv.) (maximal dose  $8\mu\text{g/kg/min}$ ), until criteria were fulfilled or unacceptable side effects appeared. Systemic arterial blood pressure (SBP) was monitored. In case of unacceptable RHH despite sodium nitroprusside testing, optimization of oral medication was performed, including diuretics iv. or introduction of IABP before a new vasodilation test was performed<sup>45</sup>.

### **6.3. Immunosuppressive protocols.**

#### **6.3.1. CNI in combination with steroids and MMF/azathioprine.**

**From 1983 until 2002;** CsA and AZA were started orally 2-4 hours preoperatively; CsA at a dose of 4 or 6 mg/kg according to estimated glomerular filtration rate (eGFR) below or above 50 ml/min/1.73m<sup>2</sup>, and AZA 4 mg/kg. Next dose of both agents were administered orally 12 hours postoperatively and continued to achieve blood concentrations as in table 1 below.

**2002-2009;** Premedication was restricted to 1 g MMF. CsA was administered iv. as a 4 hour infusion immediately postoperatively and continued twice daily until oral medication was possible and then continued as in table 1.

**2009-2010;** Induction therapy with basiliximab was introduced to patients with eGFR below 50 ml/min/1.73m<sup>2</sup>. First dose was administered 4 hours preoperatively and second dose at day four post-HT. CsA was initiated orally first or second day depending on renal function. Patients with eGFR  $\geq$  50 ml/min/1.73m<sup>2</sup> initiated CsA and MMF preoperatively as before 2009.

Table 1. History of immunosuppressive protocols at Oslo University Hospital Rikshospitalet.

Agent	Time	CsA pre/per op	C <sub>0</sub> < 1 mo.	C <sub>0</sub> 1-3 mo.	C <sub>0</sub> 4-12 mo	C <sub>0</sub> > 12 mo.
CsA	1983-2002	4-6 mg/kg preop.	250-350 µg/l	150-250 µg/l	120-200 µg/l	60-120 µg/l
CsA	2002-	iv. 1mg/kg postop.	250-350 µg/l	150-250 µg/l	120-200 µg/l	60-120 µg/l
AZA	1983-2002	4 mg/kg	50-150 mg/d	50-150 mg/d	50-150 mg/d	50-150 mg/d
TAC	2000-	NA	8-10 µg/l	6-8 µg/l	6-8 µg/l	4-6 µg/l
MMF	2002-	1 g	1-3 g/d	1-3 g/d	1-3 g/d	1-3 g/d
EVE+CsA*	2007-	NA	4-6 µg/l + 125-175 µg/l	4-6 µg/l + 50-125 µg/l	6-8 µg/l + 100-25 µg/l	3-5µg/l+25-40 µg/l
EVE*	2005-	NA	NA	8-10 µg/l	6-10 µg/l	5-7 µg/l

NA: not applicable. CsA:cyclosporine A. AZA: azathioprine. TAC: tacrolimus. MMF: mycophenolatemofetil.

EVE: everolimus. \*In combination with MMF/AZA and steroids.

During all periods methylprednisolone was administered intravenously perioperatively, continued postoperatively as oral prednisolone at a dose of 0.2 mg/kg, and gradually reduced to a maintenance dose of 0.1mg/kg.

### 6.3.2. Everolimus without concomitant CsA.

Within the first year after HTx, EVE without CsA was introduced with a dosage of 2.25 mg twice daily, while in long term survivors EVE was initiated 2.0 mg twice daily. Blood concentration levels were targeted as in table 1. MMF was reduced in case of side effects due to increased exposure after CNI withdrawal. Steroids were continued as before switch. In case



of major surgery, EVE was replaced by CsA one week before and reinstituted two weeks after the operation or until proper wound healing was accomplished<sup>46</sup>.

### **6.3.3. Low dose Everolimus and low dose CsA.**

In the NOCTET trial, EVE was initiated overnight with a starting dose of 0.75 mg b.i.d. and with a target concentration in the range 3-8 µg/l. After an initial period with a significant increase in infections in the EVE arm, a reduction in concentration to 3-6 µg/l was recommended by the data safety committee. Upon initiation of EVE a parallel reduction of CNI dosage was performed to achieve a trough level reduction of 30-70 % compared to baseline, with the target of achieving a CsA trough level <75 µg/l or a TAC trough level <4 µg/l. For patients in the EVE group receiving CsA and MMF, a 25-50 % reduction in MMF dose was recommended one week after introduction of EVE with further MMF dose reduction as required. For EVE-treated patients receiving TAC, MMF treatment was to continue unchanged unless medically necessitated. MMF level measurements were performed based on the individual clinician's discretion. Concomitant medication with AZA, with or without steroid therapy, was continued according to local practice.

## **6.4. Definition of end points.**

### **6.4.1. Mortality.**

Mortality data for all patients were collected from the Norwegian population register and cause of death obtained from recipients local hospital.

### **6.4.2. Hemodynamic end points.**

The hemodynamic protocol is described under 3.2. Our end points are based on review of the literature identifying 47 studies describing 72 populations of healthy volunteers that were examined for pulmonary arterial pressure. Normal resting MAP was  $14.0 \pm 3.3$  mmHg, with the upper limit of 20.6 mmHg. A resting MAP of 18-20 mmHg was defined as a “grey zone”<sup>47</sup>. Normal value for PVR is reported 0.25- 1.5 WU<sup>44</sup>. In the context of HTx, 2.5 WU is adequate in defining what is accepted for HTx and elevated PVR in our center. ISHLT guidelines indicate 3.0 WU as acceptable level of PVR before reversibility testing is considered<sup>12</sup>.

### **6.4.3. Renal end points.**

Serum creatinine measurement is easily available and widely used at low cost. Estimated GFR was based on the MDRD formula  $[GFR (mL/min/1.73 m^2) = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})]$  (conventional units)]. Based on the definitions from the Acute Kidney Injury Network (AKIN)<sup>48</sup>, patients were divided into two groups: No-ARF (Group 1) and ARF (Group 2, 3 and 4), based on serum creatinine changes from preoperatively to 7<sup>th</sup> postoperative day. ARF patients were divided into those increasing their creatinine  $< 26.4 \mu mol/l$  or  $< 50 \%$  (Group 2), increasing their creatinine  $\geq 26.4 \mu mol/l$  or  $\geq 50 \%$  (Group 3) or in need of early dialysis (group 4). AKIN criteria represent changes in renal function within 48 hours. Serum creatinine values reported in our ARF article are based on 7th day measurements and therefore does not completely reflect the AKIN definition of change in renal function in 2 days.

**The proposed Acute Kidney Injury Network (AKIN) criteria for the definition and classification of ARF.**

Stage 1: Increase in serum creatinine  $\geq 26.2 \mu\text{mol/L}$  or increase to  $\geq 150\text{--}199\%$  (1.50- to 1.99-fold) from baseline. Urine output  $< 0.5 \text{ mL/kg/h}$  for  $\geq 6 \text{ h}$ .

Stage 2: Increase in serum creatinine to  $200\text{--}299\%$  ( $> 2.0\text{--}2.99$  fold) from baseline. Urine output  $< 0.5 \text{ mL/kg/h}$  for  $\geq 12 \text{ h}$ .

Stage 3: Increase in serum creatinine to  $\geq 300\%$  ( $\geq 3$ -fold) from baseline or serum creatinine  $\geq 354 \mu\text{mol/L}$  with an acute rise of at least  $44 \mu\text{mol/L}$  or initiation of RRT  
Urine output  $< 0.3 \text{ mL/kg/h}$   $\geq 24 \text{ h}$  or anuria  $\geq 12 \text{ h}$ .

In the NOCTET substudy, GFR was measured at baseline and at month 12 using Cr-ethylenediamine tetraacetic acid clearance or an equivalent recommended method. Patients were categorized into 3 groups (mGFR 60-89, 30-59 and 20-29  $\text{mL/min/1.73m}^2$ , respectively) according to baseline mGFR by utilizing the National Kidney Foundation Disease Outcomes Quality Initiative (NKF-K/DOQI) Guidelines<sup>49</sup>. Data regarding urine dipstick chemical analysis at baseline and one year follow-up was available for a subset of patients.

Dialysis through continuous arterio-venous hemo-diafiltration or intermittent dialysis sessions were carried out when transplant recipients showed signs of volume overload, [(RAM)  $> 12 \text{ mmHg}$ ], peripheral oedema or pulmonary congestion in combination with urinary output  $< 0.5 \text{ mL/kg/hour}$  over 24-48 hours with rapid creatinine increase by  $50 \mu\text{mol/l/day}$  over 2 days, serum creatinine rapidly rising above  $200 \mu\text{mol/l}$ , or generally accepted criteria for dialysis.

## **6.5. Statistical analysis.**

Statistical analyses were performed with SPSS statistical software version 13.0 (SPSS inc. Chicago, IL). Results are presented as mean values  $\pm$  standard deviation or median with interquartile range. Student's t-test was used for normally distributed continuous variables and Mann-Whitney test for other continuous variables. Categorical variables were compared using the chi-square test. Group comparison over time was made using one-way Anova. A two-sided  $p < 0.05$  was considered statistically significant. Kaplan-Meier analysis with log-rank test was performed to compare the number of events in the two groups. Cox proportional hazard analysis, including all variables significant upon univariate analysis ( $p < 0.05$ ), was performed to determine the independent variables for all-cause mortality and graft loss. All retransplants (n=11) were in this context considered as deaths. Propensity score, to identify variables at different time periods was used including three different methods (binary logistic, stratification, matching).

## **6.6. Ethical consideration.**

As our HTx database is approved by the The Institutional Review Board at our hospital as well regional ethical committee, no ethical dilemmas were considered in paper 1 and 2. The NOCTET trial [Clinicaltrials.gov (unique registration number NTC00377962)] was approved by the national ethics committee in respective countries and the study was carried out in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, applicable local regulations and the Declaration of Helsinki.

For the recipients in paper 3, development of a local protocol was performed to offer an alternative immunosuppression to those recipients with severe renal failure caused by CNI

exposure. For the long term users the development of renal failure over the last years and a possibility of ending in dialysis within 1-2 years were considered as a proper reason to discontinue CNI medication<sup>22</sup>.

All patients were informed about renal failure as a predictor in HTx. Information about the possibility of CNI avoidance was given in a consultation between a dedicated study physician and each potential participant. Patients were informed about possible side effects and were free to accept the conversion from CNI to EVE. They were also free to withdraw at any time point and return to CNI treatment.

## **7. Summary of papers.**

### **Paper 1. Pulmonary hypertension in heart transplantation: discrepant prognostic impact of preoperative compared to one year postoperative right heart hemodynamics.**

We wanted to evaluate the natural course and impact of pre- and post-operative right heart hemodynamics (RHH) in 500 consecutive HTx recipients. The main findings in the study were: (i) RHH before HTx are not predictive for survival after HTx as long as patients with severe PH are reversed by vasodilator administration, (ii) repeated RHC, while waiting for HTx seems to be of limited value, (iii) hemodynamic variables improve to near normal values within the first 2 postoperative weeks in most HTx recipients, while (iv) RHH indicating PH one year after HTx are strong independent risk factors for long term survival.

### **Paper 2. Acute renal failure early after heart transplantation: risk factors and clinical consequences.**

We wanted to examine the incidence, risk factors and implication of acute renal failure (ARF) after HTx. Our main findings were that ARF occurred in 12 % after HTx. ARF was associated

with increased short-term mortality and correlated to the degree of ARF based on AKIN criteria. ARF was not a risk factor for later need of dialysis or kidney transplantation.

The strongest determinant of developing ARF was CsA given intravenously.

### **Paper 3. Benefit of early conversion from CNI-based to everolimus based immunosuppression in heart transplantation.**

We wanted to evaluate the effect of CNI elimination and EVE introduction in HTx recipients with severe renal failure early and late after HTx to evaluate the potential in renal improvement. Our results suggest that conversion from CNIs to EVE is safe and demonstrate substantial improvement in renal function among those converted within the first year after HTx. In long term survivors with longer exposure to CNIs there was no improvement, but possibly stabilization, in renal function.

### **Paper 4. Improvement in renal function after everolimus introduction and calcineurin inhibitor reduction in maintenance thoracic transplant recipients: the significance of baseline GFR.**

In this sub-study of the NOCTET trial we wanted to evaluate the potential improvement in renal function among recipients with different stages of renal failure. Our findings were that introduction of low dose EVE and parallel CNI reduction significantly improved renal function amongst maintenance TTx patients with pre-existing moderate and advanced, but not mild renal failure. However, pharmacological intervention with EVE needs to be considered at a relatively early stage after transplantation as potential improvement seems to be more effective during the first years after TTx.

## **8. Discussion.**

Despite a rather intense research and focus on mechanical circulatory support, the field of HTx has continued to evolve. Progress has been made over the last years to minimize complications caused by immunosuppression, otherwise reducing both quality of life and increasing morbidity and mortality. Successful HTx relies on a thorough candidate selection, donor availability and preservation, perfect surgery, close follow up and patient compliance. Our program is based on traditionally accepted guidelines, but there are few randomized trials to support the scientific evidence in HTx.

### **8.1. Pulmonary hypertension in heart transplantation.**

#### **8.1.1. Pulmonary hypertension before heart transplantation.**

PH is reported in up to 80 % of HF patients, depending on the definitions<sup>14</sup>. The number of patients with preoperative MAP > 20 mmHg among our HTx recipients is 84%, confirming that the sickest candidates selected for HTx also have the highest pulmonary pressures<sup>18</sup>. The presence of PH is associated with worse outcomes in non-transplanted HF patients, regardless of ejection fraction and stage of HF<sup>13-15</sup>, and development of RV failure post-HTx prognosis is further aggravated by irreversible PH<sup>50;51</sup>. Presence of PH is probably an evidence of more disease burden and often complicated multi-morbidity.

Our patients with the most severe PH were males, diabetics and with a background of ischemic heart disease, risk factors that vary in previous reports<sup>3;5;14;18</sup>. They also had increased systemic blood pressure, an additional afterload burden causing more intense backward failure and increased pulmonary pressures<sup>52;53</sup>. Age was not a predictor for PH neither before nor after HTx, even though pulmonary pressures increase with age both at rest

and during exercise in healthy controls<sup>14;54</sup>. In contrast to a small previous report, smoking was not a predictor for PH suggestive that heart failure hemodynamics as the driving force and more prominent than changes caused by age and smoking<sup>55</sup>.

Our reversibility protocol for PH is relatively aggressive and a very limited number of patients with severe PH are non-responders to vasodilation testing compared to other reports. Importantly, responders to vasodilatation faced similar prognosis as recipients with acceptable pressures. Our results therefore challenge the opinion of a poor prognosis for those with the highest pressures responding to vasodilation. No absolute PVR cut off is reported in regards to post-HTx survival, but rather replaced by the recognition of elevated pulmonary pressures and PVR as incremental risk factors with increasing values<sup>11;17</sup>. However, patients with irreversible PH can in the present era be candidates for LVAD treatment, allowing otherwise suitable HTx candidates to be placed on the waiting list<sup>56-58</sup>. With third generation continuous, non-pulsatile LVAD they face the same prognosis post HTx as patients with acceptable RHH<sup>11;59</sup>.

HF changes in the pulmonary circulation usually precede changes in systemic hemodynamics. Patients with chronic LV dysfunction may develop PH due to a backward failure that is largely reversible with normalization of LV filling pressures. However a sustained and excessive exposure to pulmonary venous hypertension leads to functional and structural changes in the pulmonary vasculature, initially in the capillaries and later in the arterioles and arteries<sup>60</sup>. The exact relation between PH and structural changes in the pulmonary circulation is not fully understood and is probably individual due to response and sensitivity to changes in endothelial nitric oxide production, decreased expression of angiotensin converting enzyme, increased levels of endothelin and proinflammatory cytokines<sup>52</sup>. While permissive genotypes could possibly explain these variations, studies in this field have yet to be performed<sup>61</sup> (fig 2).



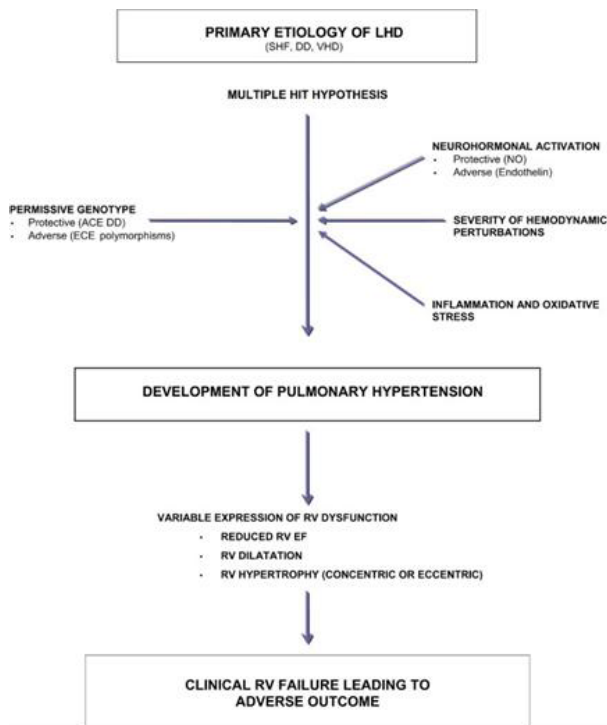


Figure 2. Model for development and consequence of pulmonary hypertension. Reproduced with permission from Park and Mehra. J Am Coll Cardiol. 2012

A close relationship between pulmonary pressures and PCW at baseline and a parallel reduction during vasodilation test suggests that left heart filling pressures is the main component of PH in HF and a simple response to chronically elevated left-sided filling pressures coupled with dynamic interaction between the two ventricles<sup>12;53</sup>. On the other hand, PCW decreased more than MAP and of the 135 patients in need of vasodilation, 35 % increased their TPG, and 16 % increased TPG to more than 20 mmHg as an indication of structural changes in the precapillary vasculature bed after longstanding HF in a subgroup of patients<sup>15</sup>.

### **8.1.2. Repeated catheterizations while waiting for heart transplantation.**

Our waiting list for HTx is relatively short compared to other countries. In our cohort, RHH were stable while waiting for HTx. One hundred patients were recatheterized in the waiting period. Most of the patients improved their RHH but 10 out of 70 patients went from acceptable pressures to need of vasodilation test. Most important, no patients developed fixed PH, and none had to be taken off the list. The data on RHH while waiting for HTx is scarce and guidelines for patients in need of reversibility test are based on level of evidence 1C<sup>12</sup>. Our strategy with quarterly visits in addition to a close cooperation with local “heart failure clinics” may reduce the number of patients deteriorating and who become ineligible for HTx. We also have a relatively low mortality rate (< 5%) on the waiting list supporting our arguments.

### **8.1.3. Pulmonary hypertension after heart transplantation.**

After HTx, when left sided filling pressures were lowered, the pulmonary venous congestion decreased with secondary reduction in pulmonary pressures<sup>62</sup>. Pulmonary pressures showed a decline to near normal values within few weeks post HTx. Compared to those with acceptable pressures (Group 1), Group 2, in need of vasodilation, had a more impressive improvement than Group 1, but some recipients did not normalize pulmonary hemodynamics despite normalization of PCW pressures. Reduction in postoperative PH is previously reported with variable time to normalization<sup>18;63</sup>.

Increased MAP, as well as other hemodynamic parameters evaluated post-HTx were strong predictors of late mortality. PCW and RAM were the only hemodynamic parameters

similar between two groups with post-HTx MAP above or below 20 mmHg. We suggest that these patients' additional risk may be caused by structural changes in the pulmonary vasculature, not reversed by LV unloading<sup>64</sup>. MAP, CI and TPG were robust predictors for mortality also after 6 months, 2 and 3 years (data not shown) as well as PVR 2 weeks post-HTx, shown in another study from our hospital (in press) (ISHLT 2012 abstract 166)<sup>65</sup>. Despite lack of data, medication proven efficacious in precapillary PAH patients, such as phosphodiesterase inhibitors and endothelin receptor antagonists, are being used to manage other forms of PH<sup>63</sup> (ISHLT 2012 abstract 131). Although this approach may be justified in carefully selected patients with TPG out of proportions and reactive PH, medication may be ineffective or even harmful for most of HTx recipients with PH<sup>66;67</sup>. So far no therapeutic option has been validated to improve the prognosis of those with elevated MAP after HTx, reflecting the lack of focus on these recipients at risk<sup>15;22</sup>.

PH is fairly common shortly after HTx<sup>68;69</sup>. Risk factors for developing MAP > 20 mmHg at one year were unfavorable preoperative hemodynamics in addition to male donor organ. Correlation between preoperative and at one year post-HTx MAP was poor ( $r = 0.236$ ), reflecting the multitude of factors influencing postoperative RHH. Male donor organ as risk factor was stronger for women than for men and there were a higher proportion of male organs in the group with elevated MAP at one year post-HTx.

## **8.2. Renal function before and after HTx.**

Heart failure with reduced CO often aggravates renal disease or initiates renal impairment due to hypoperfusion<sup>3</sup>. Activation of the RAS system leads to salt and water retention that also paradoxically worsen the cardiac function and leads to venous congestion and a reduction in tissue perfusion<sup>70</sup>. Renal function is a strong independent predictor of long-term adverse

outcome in HF patients, as well as in HTx recipients<sup>35;38;71;72</sup>. New research and treatment modalities target renal protection both early and late after HTx<sup>34</sup>.

### **8.2.1. Assessment of renal failure.**

ARF is defined in 3.4.3. The true definition of acute kidney injury (AKI) is an abrupt reduction in kidney function during 48 hours<sup>48</sup>. Dialysis was usually initiated before a 2-3 times increase in baseline creatinine was achieved, usually due to reduced diuresis and volume overload. An ideal definition of ARF in our HTx recipients would also be the change in renal function within 48 hours (AKIN criteria), of which we do not have the details to assess. Studies report a multitude of factors influencing renal function early postoperatively<sup>73;74</sup>. Most reports define ARF as need for dialysis. Traditional risk factors for dialysis are reduced pre-HTx GFR, need of preoperative prolonged inotropes or mechanical circulatory support, complicated, prolonged or redo surgery and older recipients<sup>75;76</sup>.

Independent of time period, the incidence of ARF was steady at 18 % before introduction of iv. CsA, when the need of dialysis increased up to an incidence of 23 % after 2002. Dialysis is a more definite end point, but depends on the physician's judgement and ability to interact when renal function declines. Considerable variations in mortality for HTx ARF patients in dialysis probably reflect different criteria for initiation of dialysis. A Finnish study reports an incidence of dialysis of 25 % despite induction therapy, but incidences upto 32% are reported for HTx patients with impaired pre-HTx renal function. Otherwise the need for dialysis varies between 6 % and 13 %<sup>73;75-77</sup>.

In paper 3 and 4, renal failure was classified according to the National Kidney Foundation Disease Outcome Quality Initiative.  $\text{GFR} < 30 \text{ ml/min/1.73m}^2$  is classified as severe renal failure, while  $\text{GFR}$  between 30 and  $59 \text{ ml/min/1.73m}^2$  is defined as moderate

reduction. Most large randomized trials assess renal function with mGFR at baseline and follow up. In clinical work eGFR and creatinine are surrogate markers of renal function, reported equally as markers of prognosis<sup>36;38</sup>. In paper 4, mGFR and eGFR correlated with a  $r=0.513$ ,  $p<0.001$ , and statistical analysis with eGFR would not significantly have altered the results in this sub-study, nor in the main study.

In paper 4, as in the landmark CONVERT trial, an mGFR improvement of 5 ml/min/1.73m<sup>2</sup> was chosen as a cut-off value of significant improvement in renal function after introduction of EVE in renal TX<sup>78</sup>.

Table 2. Stages of chronic kidney disease.

Stage	Description	GFR (ml/min/1.73m <sup>2</sup> )
1	Kidney damage with normal or elevated GFR	≥ 90
2	Kidney damage with mild reduction of GFR	60-89
3	Moderately reduced GFR	30-59
4	Severely reduced GFR	15-29
5	Kidney failure	<15 or dialysis

Chronic kidney disease is defined as either kidney damage or GFR < 60 ml/min/1.73m<sup>2</sup> >3 months including markers of damage in blood, urine test or imaging studies.

Proteinuria is an early sign of renal disease and often represents a more general vascular damage. Proteinuria is associated with impaired renal prognosis and increased cardiovascular mortality even with a normal creatinine levels at time of measurements<sup>79</sup>. It has been demonstrated that PSI agents can increase glomerular pressure and contribute to proteinuria and progression of pre-existing kidney damage<sup>80;81</sup>. PSI agents may also cause glomerulopathy or tubulointerstitial disease related to interference with protein endocytosis in

tubular epithelial cells<sup>19;82</sup>. Absence of proteinuria is reported as a discriminator for success when introducing EVE to reduce CNI and improve renal function<sup>83;84</sup>.

No internationally accepted protocol on proteinuria has been published for HTx recipients in contrast to renal Tx<sup>8</sup>. In our institution initiation of EVE can be performed if protein/creatinine ratio < 50 mmol/ml. Patients with ratio between 50-100 mmol/ml are candidates for pre-treatment with ACE inhibitors or ARBs<sup>85;86</sup>. Recipients with ratio > 100 mmol/ml are usually not candidates for EVE therapy, while a low dose EVE in combination with low dose CsA might be an alternative<sup>80</sup>. In our ARF study we did not quantify proteinuria. In paper 3, proteinuria was only a clinical dilemma in a few patients in Group 2. In paper 4, proteinuria was not a clinical problem in a treatment strategy with low dose EVE. In low dose EVE or when introduced early post-HTx, proteinuria does not seem to be a problem, but need attention in long term recipients or in recipients with underlying renal disease<sup>8;83;87</sup>. Whether proteinuria induced by PSI is a negative predictor with consequences similar to proteinuria in native renal disease is unknown. CNI as a vasoconstrictor may unmask existing proteinuria in renal damage instead of proteinuria being induced by introduction of PSI. Manifest proteinuria has been a discriminant factor for successful introduction of EVE in renal, lung and heart Tx, and probably reflects renal disease beyond CNI induced vasoconstriction<sup>33</sup>. Factors reported to be associated with increased proteinuria after solid organ TX are poor renal function, loading dose with sirolimus, diabetes and histological abnormalities<sup>33</sup>.

### **8.2.2. Determinants of acute renal failure.**

The most important factor for development of ARF was administration of iv. CsA early postoperatively. No HTx study has previously assessed renal function based on CsA administration and blood concentrations early postoperatively. Guidelines on iv.

administration of CsA concerning indication, once or twice daily, duration of infusion or concentration levels do not exist in the early postoperative setting<sup>22</sup>. Vallentine et al demonstrated a better rejection profile after two six hour infusions compared to continuous 24 hour infusion without considering the deleterious effect on renal function<sup>21</sup>. As high CsA peak concentration provides better protection from rejection, the side effects are greater vasoconstriction and reduced renal blood flow. Regrettably, to further demonstrate the unfortunate circumstances of iv. CsA administration, we did not measure  $C_{max}$ .

CsA alters the balance between vasodilator and vasoconstrictor prostaglandins and increases renal arteriolar resistance and mesangial cell contraction<sup>88</sup>. The intrarenal RAS system may also contribute to both nephrotoxic and hypertensive effects of CsA<sup>8</sup>. Prolonged CsA therapy causes chronic renal failure with a variety in severity. Older age, sex, underlying renal disease, different genotypes influencing CYP3A activity in renal cells and years with elevated CsA AUC predispose to chronic CsA nephrotoxicity<sup>21;89;90</sup>. Long term decreased renal blood flow induces glomerular sclerosis, with interstitial fibrosis and thickening of capillary basement membrane<sup>40</sup>. Once chronic renal failure is established decreasing CsA concentration or drug withdrawal will not necessarily improve renal function<sup>91</sup>.

Recipients who developed ARF were older and received older donor hearts, a traditional risk factor of increased mortality<sup>10</sup>. Aging donor hearts implicate vulnerable initiation after cardioplegia and need of more inotropic agents to maintain adequate hemodynamics.

Preoperative elevated RAM (CVP) was a predictor for dialysis and increased CO was an independent predictor for ARF. Patient in need of dialysis had lower CO and increased pulmonary pressures representing unfavourable RHH and reduced renal perfusion<sup>72</sup>. In HF patients, volume overload/ high RAM increase ventricular filling pressures and myocyte stretch<sup>44</sup>. Increase in force generation enables the healthy heart to eject the additional venous

return thereby increasing the stroke volume. For the patients in need of postoperative dialysis, hemodynamic parameters probably reflect the lack of contractile reserves and force velocity to maintain adequate circulation and renal perfusion. As both hemodynamic parameters and reduced pre-HTx GFR are predictors of ARF and dialysis post-HTx, it is once again a reminder that the cardiorenal syndrome is proven essential in HF and continuous importance in the HTx recipient<sup>92;93</sup> (fig 3).

In paper 3, all patients in Group 1 had postoperative ARF partly due to administration of CsA, as renal function improved markedly when our short term recipients had their CsA withdrawn and replaced by EVE. Paper 3 did not emphasize on predictors for the rapid post-HTx increase in creatinine that all patients experienced. Additional mechanisms for CsA nephrotoxicity is likely in the per- and post-HTx setting, but CsA withdrawal significantly improved renal function that has remained stable with an extended follow up of 4.5 years. There was no control group to evaluate the natural course of renal failure for patients on traditional CsA medication, but a progression in renal impairment is described and experienced by all HTx physicians<sup>35</sup>. In a histopathological study of renal failure, a diverse spectrum of diagnosis indicate that long term recipients are at risk of nephrosclerosis and diabetic nephropathy as well as CNI toxicity<sup>43;94</sup>. These findings suggest a possible benefit in the form of stabilization of kidney function after a switch to EVE also among the long term users in Group 2.



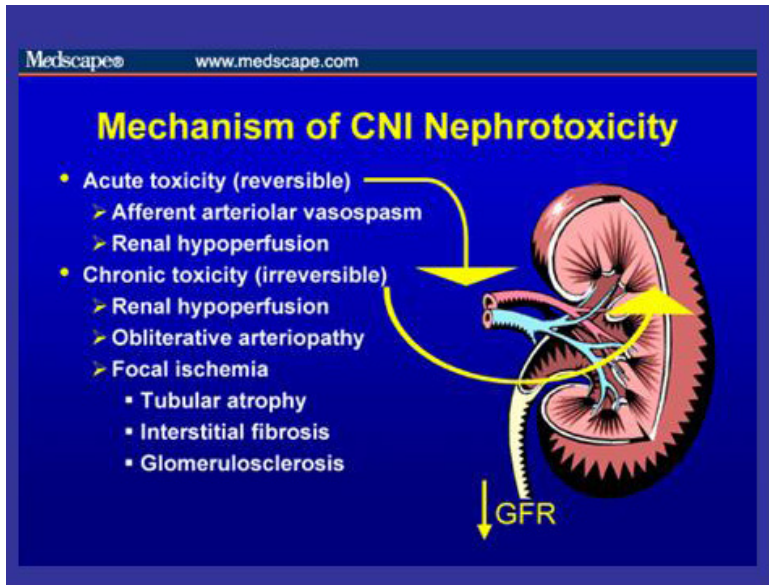


Figure 3. Mechanisms of CNI nephrotoxicity.

### 8.3. Immunosuppression in HTx.

Our immunosuppressive protocol has traditionally been CsA based with conversion to TAC in case of CsA side effects such as gingivitis, hirsutism or repeated rejections<sup>24</sup>. After introduction of TAC in 1993, inferior results were initially reported compared to the new microemulsion CsA Neoral. Later studies have reported similar survival rates, even though a review from 2010 with meta-analysis were in favor of TAC<sup>20;24;95;96</sup>. ISHLT report increasing use of TAC, but more long term users switch from TAC to CsA based immunosuppression than vice versa<sup>5</sup>. Prolonged release formulation and mono-therapy with TAC has been proven safe, but without long term follow up<sup>97;98</sup>.

In the later years EVE has been increasingly suggested used due to less nephrotoxicity. Reports on EVE in de novo HTx demonstrate variable results and randomized trials are ongoing. I will discuss the administration form of CsA in addition to the introduction of EVE

with focus on minimilization or elimination, blood concentration levels and timing for therapy intervention.

### **8.3.1. Early postoperative peroral or intravenous CsA.**

Our traditional protocol introduced po CsA preoperatively to maintain adequate CsA levels early post-HTx. CsA is lipophilic and absorbed in the upper gastrointestinal tract, with variable absorption the first days post-HT. The newer microemulsion form of CsA (Neoral) has more favorable outcome than the old oil formulation and greater bioavailability  $[(AUC_{oral}/AUC_{iv}) \times (Dose_{iv}/Dose_{oral})]$  that is approximately 30 % of iv. administration.<sup>20;21;89;99</sup> Iv. CsA resulted in a higher proportion of ARF. Unfortunately we do not have sufficient details to correlate iv. and po. CsA concentrations with the rejection profiles of the two subgroups of ARF. In the literature, iv. administration of CsA is also called induction therapy<sup>23</sup>. As newer induction agents have been developed, our experience with iv. CsA should indicate that this administration form should be limited to a minimum early postoperatively. ISHLT reports shows that induction therapy does not provide additional rejection efficacy compared to a non-induction strategy, results that contrast smaller reported studies<sup>5;20;100</sup>. As renal sparing strategy, induction therapy is proven effective and its use is widespread<sup>22;101</sup>. Guidelines recommend relatively high doses of CNI early post-HTx, especially from US centers. Different  $C_0$  recommendations complicate collaborate studies in European and US centers<sup>22;102</sup> (ISHLT 2012 abstract 201).

### **8.3.2. CNI reduction and everolimus introduction.**

Two major studies, including 1250 recipients, have been performed combining EVE and CNI in de novo HTx recipients. Both studies have replaced MMF or AZA with EVE and the blood

concentrations of CNI have remained rather high and renal protection has not been observed<sup>103</sup> (ISHLT 2012 abstract 201). In other de novo studies, combination of CsA and EVE have not provided any renal improvement compared to CsA standard therapy in addition to MMF and steroids<sup>104;105</sup>. No renal protection was reported in an open de novo triple arm study with combination of TAC and sirolimus, compared to TAC or CsA in combination with MMF and steroids<sup>95</sup>. As a consequence of non-successful trials, a strategy for early introduction of EVE and CNI reduction has not been established and a substitution of PSI for MMF in addition to standard CNI concentrations are not recommended due to risk of enhanced CNI nephrotoxicity<sup>22</sup>.

Studies including long-term HTx recipients are smaller and have not established a consensus on combination therapy of CNI and EVE. In non-randomized reports, CNI reduction or withdrawal with EVE replacement partly preserved renal function<sup>27;83;106</sup>.

As CsA and EVE share the same glycoprotein in kidney tissue, concomitant therapy possibly potentiates renal toxicity. Co-administration of CsA microemulsion increased AUC for EVE concentrations by 168 %, but not vice versa, and a substantial CNI reduction has to be achieved before renal protection is observed<sup>89</sup>. Reports are often complicated with high frequency of adverse events and withdrawals due to overimmunosuppression<sup>32;105</sup>.

The NOCTET trial included 282 TTx recipients more than 1 year after transplantation. Of 190 HTx recipients, our center included 78 patients. Paper 4 reports results from both HTx and lung Tx. Results were mostly comparable for the two groups, although slightly in favor of HTx in terms of beneficial effect on renal function. The following discussion will focus on HTx.

The NOCTET trial demonstrated that EVE introduction and parallel CNI reduction significantly improves renal function in maintenance TTx patients with an overall difference of 5.1 ml/min/1.73m<sup>2</sup>. However, there was a significant interaction with pretreatment renal

function, evidenced by a mGFR improvement of nearly 7 ml/min/1.73m<sup>2</sup> and 5 ml/min/1.73m<sup>2</sup> in patients with baseline severely or moderately reduced mGFR, respectively. In contrast, no improvement was observed in patients with mGFR > 60 ml/min/1.73m<sup>2</sup>. Previous guidelines do not recommend a switch to EVE therapy amongst patients with severe pre-existing renal damage, an opinion challenged by our results and in a recent German review<sup>23;101;107</sup>.

Paper 4 demonstrated that this strategy is clinically relevant as 66% of patients with the most advanced CRF had an improvement of >5 ml/min/1.73m<sup>2</sup> with EVE therapy as compared to 54% of patients with less severe pre-existing renal damage. It should be noted that the group with the most deranged renal function had higher CsA baseline concentrations than the two other groups. A further potential for renal improvement would probably be possible with CsA adjusted to a lower range as a correlation between CsA reduction and renal function is reported<sup>106</sup>. The correlation between CNI reduction and mGFR improvement was only modest suggesting also other mechanisms contributing to improvement in renal function<sup>108</sup>. As improvement was limited to patients transplanted less than five years, intervention should be initiated before renal fibrosis is established.

After an initial period with a significant increase of infections in the EVE arm, a reduction in EVE blood concentration from 3-8 to 3-6 ng/mL was recommended by the control committee due to overimmunosuppression also experienced in other studies<sup>83</sup> (ISHLT 2012 abstract 201). Studies intending to improve renal function, de novo or in long term recipients, with concomitant EVE introduction report CsA concentrations that are too high to significantly improve GFR<sup>83;105</sup>. Poor adherence to planned CsA targets, the concern of CsA underexposure, distrust to EVE as rejection prophylaxis and risk of rejection in the early post-HTx setting result in both EVE and CsA concentrations with secondary renal toxicity<sup>105</sup> (ISHLT 2012 abstract 201).

After increasing experience with combination therapy, CsA is in long term recipients in our center now aimed at 25-40 µg/l in combination with EVE 3-5 µg/l in addition to MMF and steroids. As interaction and low doses of both drugs often make target concentration hard to achieve, different dose ( $\pm 25$  mg) of CsA every second day is possible or CsA blood concentration in non-detectable range ( $< 25$  ng/ml), with a  $C_2$  concentration  $> 180$  ng/ml and EVE 4-6 ng/ml is a second alternative in order to reduce CsA substantially. Our experience with EVE in combination with TAC is limited<sup>22</sup>.

### **8.3.3. CsA elimination and everolimus introduction.**

Few reports exist on PSI without concomitant CsA after HTx, and most reports are on long term survivors<sup>27;28;31;109;110</sup>. Improvement in renal function is reported, but high frequency of adverse events and withdrawals complicates conclusions. Complete discontinuation of CNI replaced by sirolimus introduction was evaluated in the “Heart Spare the Nephron Study” intended to include 580 HTx de novo recipients. (Hunt, 2007 ISHLT). The study was stopped after 4 out of 7 sirolimus treated recipients experienced rejection  $\geq 3A$ .

In paper 3, Group 1 experienced a rapid improvement in renal function. Sixteen HTx recipients 5.5 months after HTx, including five patients ongoing in dialysis and 15 long term recipients (8 years post-HTx) were switched overnight from CNI to EVE based immunosuppressive therapy. Our results suggest that early CsA withdrawal is safe with preserved renal function in long term, also reported in a 5 years follow up of 15 de novo CNI free recipients<sup>111</sup>.

In our hospital the RENAL study (renal Tx) and the SCHEDULE study (HTx) introduced EVE in combination with MMF, steroids and CsA de novo and eliminated CsA after 7-11 weeks post-HTx, compared to a traditional CNI medication. In the RENAL study

an improvement in mGFR during 12 months follow up was observed despite an increased rejection rate and a rather large proportion of drop out patients (in press). The SCHEDULE study will complete a 12 months follow up at the end of 2012.

In paper 3, long term recipients with CsA elimination 8 years after HTx (Group 2) did not improve their renal function. With a wide interquartil range, the median creatinine/eGFR was unchanged suggesting that established renal fibrosis is irreversible to CsA withdrawal. Recipients had histories of diabetes (20 %), general atherosclerosis (53 %) and hypertension (73 %), suggesting that also other mechanisms contribute to renal failure<sup>43</sup>. Although improvement was not observed, their renal function stabilized after years of deterioration and further renal impairment may have been delayed by the intervention. In contrast to our results, renal improvement in long term recipients is reported. This may be explained by earlier intervention and a renal function better than our patients before switch<sup>28</sup>.

Timing of CNI elimination early after HTx is somewhat controversial. Guidelines have recommended possible CNI elimination 6 and 12 months after HTx<sup>22;23</sup>, while in renal Tx recommendations suggest early conversion between 2 to 6 months. Due to the early CNI nephrotoxicity, “there is an obvious advantage for reducing CNI exposure early post-transplant”<sup>8</sup>. In general there is no obvious reason to accept different immunosuppression strategies for the two organs, besides the penalty of graft loss is greater in HTx than the need for recurrent dialysis in renal Tx recipients. As EVE may not have the same immunosuppressive power as CNIs<sup>112</sup>, the cutting edge of EVE therapy is whether we are willing to accept an increased risk of milder rejections with the potential benefits of EVE therapy: improved renal function, less CMV infection, less development of CAV and less development of cancer. Results from the SCHEDULE trial and other ongoing studies will hopefully give us some answers that will have future impact on immunosuppressive strategies and recommendations.

Switch from CsA to EVE can be performed stepwise or as an overnight conversion. Overnight switch is easy to perform and interpret and is uncomplicated in long term recipients. In a stepwise protocol, interaction between the two drugs requires experience to avoid oscillations in blood concentrations and either risk of rejection or renal failure. Shortly after HTx a stepwise protocol may be safer in experienced hands and with lower risk of rejections with mandatory biopsy surveillance.

In Group 1, rejections occurred in patients shortly after the switch and with the shortest time since HTx. Our rejection incidence is favorable compared to previous reports on early CNI freedom<sup>31;109</sup>. Early CNI freedom resulted in increased rejection rates in the RENAL study compared to standard CsA based treatment. Alternative T-cell activation by different signal mechanisms may increase rejection risk and suggest that a combination of CNI and EVE is required to induce synergistic protection for rejection early postoperatively. Timing of CNI elimination and EVE introduction therefore depends on clinical impact and prognostic importance of CNI side effect vs. rejection risk of the patients and tolerability of higher EVE doses. As the immunosuppressive armamentarium is expanding, each patient is now able to have an immunosuppressive strategy especially designed for the individual patient at risk.

Representing a potential improvement for patients, successful individualization of a growing number of immunosuppressive drugs will demand increased awareness and insight from physicians dealing with organ transplantation.

## **9. Conclusion.**

This thesis has investigated the role of hemodynamics before and after HTx in addition to renal failure secondary to nephrotoxic immunosuppression. The following conclusions can be drawn:

1. Despite a limited number of patients with irreversible PH, RHC before HTx is important to identify patients at risk, but repeated RHC is only needed for patients with severe PH. Pulmonary hemodynamics improve early after HTx, but unfavorable post-HTx RHH is predictive for survival.
2. ARF occurred in 12 % of HTx recipients, but increased after introduction of iv. CsA. ARF was a predictor for short term mortality, but not for the development of chronic renal failure.
3. Early conversion from CNI to EVE based immunosuppression was safe and improved renal function in contrast to long term users in whom renal function was unchanged.
4. Low dose EVE and CsA improved renal function in TTx recipients with moderate and severe renal failure when EVE was introduced within 5 years after transplantation.



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