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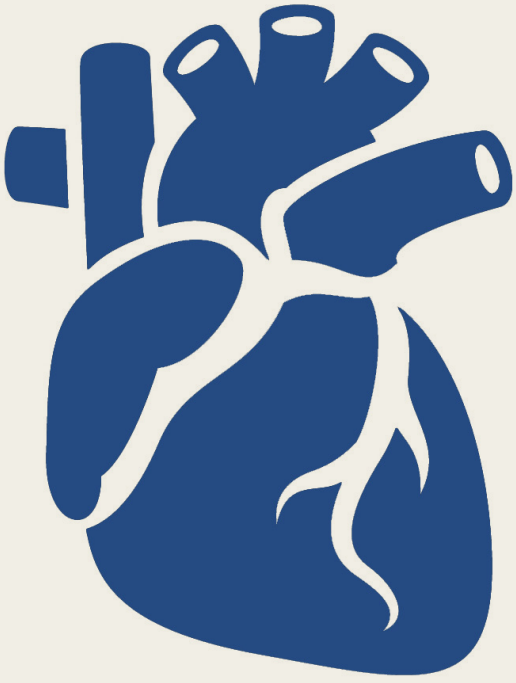

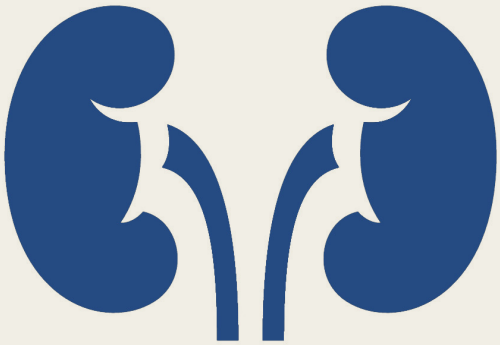

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Carl Söderlund, MD

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Title and subtitle

The balance between under- and over-immunosuppression after heart transplantation with emphasis on acute cellular rejection and chronic kidney disease – Experiences from Skåne University Hospital in Lund 1988-2010

Abstract

Background and aims:

Heart transplantation (HT) constitutes the ultimate treatment choice for end-stage heart failure. Following HT, patients require life-long immunosuppressive treatment to prevent allograft rejection. Although survival has steadily increased over the last few decades, issues related to under- and over-immunosuppression remain common and continue to represent one of the most important limiting factors for long-term outcome. This important act of balance is also a major reason for the debate on whether or not newly available generic immunosuppressants, which may provide economic advantages, should be introduced.

HTs have been performed at Skåne University Hospital in Lund (SUS-Lund) since 1988. Since the beginning of the transplant program, patients have undergone extensive follow-up including renal function measurements using the iohexol clearance method, and endomyocardial biopsies (EMBs) to detect acute cellular rejection (ACR).

The present thesis consists of four papers. Paper **I-III** aimed to study the incidence, predictors and outcome of ACR and chronic kidney disease (CKD) after HT – two of the most relevant issues related to the balance between under- and over-immunosuppression. An additional aim was to evaluate two guideline recommended glomerular filtration rate (GFR) estimating equations in HT patients (CKD-EPI and Schwartz formulae). Lastly, paper **IV** investigated the safety and efficacy of switching to two generic immunosuppressants, namely Myfenax Teva® and Tacrolimus Sandoz®.

Methods:

All papers were retrospective in design. Paper **I-III** were based on all 215 HT patients followed at SUS-Lund 1988-2010, whereas paper **IV** concerned a smaller cohort of 55 patients.

Results:

The frequency and severity of ACR was low and decreased with time post-HT. The findings however indicated that late (>1 year) more often than early (<1 year) ACR remains undetected, and that both types of ACR influence outcome. CKD was moreover common and appeared to have a negative impact on survival. Interestingly, the results also indicated that the CKD-EPI and Schwartz formulae both overestimate GFR in HT patients and thus could lead to diagnostic delay if solely relied upon. In paper **IV**, data reassuringly indicated that switching to Myfenax Teva® and/or Tacrolimus Sandoz® several years post-HT appeared safe, at least in the short-term perspective.

Conclusions:

The present thesis, focusing on the HT population at SUS-Lund 1988-2010, provides a useful in-depth overview on the incidence, predictors, and outcome of two of the most relevant issues related to the difficult balance between under- and over-immunosuppression after HT – namely ACR and CKD. Valuable initial experience on the conversion from branded to generic immunosuppressants is also presented.

Key words: heart transplantation, immunosuppression, acute cellular rejection, chronic kidney disease, generic

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Content

Papers

The thesis is based on the following four papers, which will be referred to in the text by their Roman numerals.

I **Söderlund C**, Öhman J, Nilsson J, Higgins T, Kornhall B, Johansson L, Rådegran G. Acute cellular rejection the first year after heart transplantation and its impact on survival: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. Transpl Int 2014;27:482.

II **Söderlund C**, Rådegran G. Acute cellular rejection later than one year after heart transplantation: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. Clin Transplant 2017;31.

III **Söderlund C**, Löfdahl E, Nilsson J, Reitan Ö, Higgins T, Rådegran G. Chronic kidney disease after heart transplantation: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. Transpl Int 2016;29:529.

IV **Söderlund C**, Rådegran G. Safety and efficacy of the switch to generic mycophenolate mofetil and tacrolimus in heart-transplant patients. Clin Transplant 2015;29:619.

The following review paper forms the basis of the introduction of the thesis.

- **Söderlund C**, Rådegran G. Immunosuppressive therapies after heart transplantation - The balance between under- and over-immunosuppression. Transplant Rev 2015;29:181.

All papers are attached in the appendix with permission from each publisher.

Abbreviations

ACI	Additional clinically indicated
ACR	Acute cellular rejection
AMR	Antibody-mediated rejection
APC	Antigen-presenting cell
ATG	Antithymocyte globulin
AUC	Area under the curve
AZA	Azathioprine
BMI	Body mass index
C0 levels	Trough levels
CKD	Chronic kidney disease
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CS	Corticosteroid
CSA	Cyclosporine
CT	Computed tomography
EMB	Endomyocardial biopsy
EVL	Everolimus
GFR	Glomerular filtration rate
hba1c	Glycated hemoglobin
HT	Heart transplantation
IL-2	Interleukin-2
ISHLT	International Society for Heart and Lung Transplantation
KDIGO	Kidney Disease: Improving Global Outcomes
LHTRR	Lund Heart Transplantation Research Register

MDRD	Modification of Diet in Renal Disease
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MOF	Multiple organ failure
MPA	Mycophenolic acid
mTOR	Mammalian target of rapamycin
NFAT	Dephosphorylated nuclear factor of activated T-cells
NT-proBNP	N-terminal of the prohormone brain natriuretic peptide
PGF	Primary graft failure
SRL	Sirolimus
SUS-Lund	Skåne University Hospital in Lund
TA	Transplant assessment
TAC	Tacrolimus
TCR	T-cell receptor
TDM	Therapeutic drug monitoring
WF	Working formulation

Introduction

History at a glance

The first human to human HT

Although Dr. Norman Shumway widely is regarded as the father of heart transplantation (HT), Dr. Christiaan Barnard was the first surgeon to perform such an operation in a human. In early December of 1967, Dr. Barnard and his colleagues at Groote Schuur Hospital in Cape Town successfully removed the heart of a young woman named Denise Darvall, who had died in a car accident, and implanted it in Louis Washkansky – a 55-year-old man with severe post-infarct heart failure. Mr. Washkansky's early recovery was excellent. The achievement was published in the South African Medical Journal after only three weeks [1], but was extensively covered in media earlier than that (**Fig. 1 a-b**).

Figure 1 a (left) – b (right).
Covers of *Time* (a) and *Life* (b) magazine following the first human HT.

Despite Mr. Washkansky´s excellent early recovery, his condition worsened at around two weeks post-HT. A chest X-ray revealed pulmonary infiltrates of which Dr. Barnard and his colleagues were uncertain whether they represented pneumonia or pulmonary edema as a result of rejection of the donor heart. Mistakenly, the team treated against rejection by intensifying the immunosuppressive therapy. As Mr. Washkansky in fact had developed pneumonia, this was a detrimental decision that contributed to his death from severe sepsis at only 18 days post-HT.

Early discouraging results

Dr. Barnard´s short-lived success nonetheless inspired surgeons all over the world. During the following year, 102 HTs were performed worldwide [2]. Survival rates were however far from encouraging, with only 53%, 19% and 10% of the patients still alive after one, 12 and 24 months, respectively. The major issue was recognized as the inadequate understanding of the concept of rejection, and thereby also its diagnosis and treatment. During the following years, the number of HTs dropped significantly, as surgeons began to hesitate.

Advancements in diagnostics and therapeutics

In the early 70's, only a handful of groups continued to perform HTs. One was a group at Stanford University led by Dr. Shumway, who had invented the surgical technique which still today is standard in HT [3]. Dr. Shumway and his colleagues came up with a protocol for rejection monitoring based on clinical, electrocardiographic and echocardiographic findings, together with a new strategy for rejection treatment. By doing this, they managed to achieve improved survival rates [4-5]. However, it was not until the same group later introduced the so called percutaneous transvenous endomyocardial biopsy (EMB) in clinical practice that the postoperative care of HT patients was revolutionized [6]. With this new technique, which allowed for histopathologic rejection analysis, Dr. Shumway and his colleagues developed an improved protocol for rejection monitoring which could identify rejection even before symptoms appeared.

Not too long thereafter, another important milestone was reached when the immunosuppressive effects of cyclosporine (CSA) was reported by the Belgian immunologist Jean-François Borel in 1976 [7]. Following further research on CSA in animal models, investigators concluded that CSA was more efficient than other immunosuppressants used at that time, and still sufficiently safe [8-9].

The golden era

Dr. Shumway and his colleagues soon realized the usefulness of CSA and introduced it at their clinic in 1980. Survival thereafter steadily increased, and after five years they reached rates of 83%, 75% and 70% at one, two and three years post-HT, respectively. Inspired by this success, the interest for HT was revived and the number of HTs increased more and more.

HT today and the balance between under- and over-immunosuppression

Today, HT constitutes the ultimate treatment choice for suitable patients with end-stage heart failure who remain symptomatic and worsen despite optimal medical and surgical therapy. The number of HTs performed leveled off around 1990 due to limited availability of donor hearts. Survival has however continued to improve. This promising development can be followed through the annual reports from the International Society for Heart and Lung Transplantation (ISHLT), which include statistics based on more than 400 HT centers and 100,000 HT patients worldwide. According to one of the most recent reports [10], the median survival increased from 8.5 years 1982-1992 to 10.9 years 1993-2003, and is predicted to proceed in the same direction (**Fig. 2**).

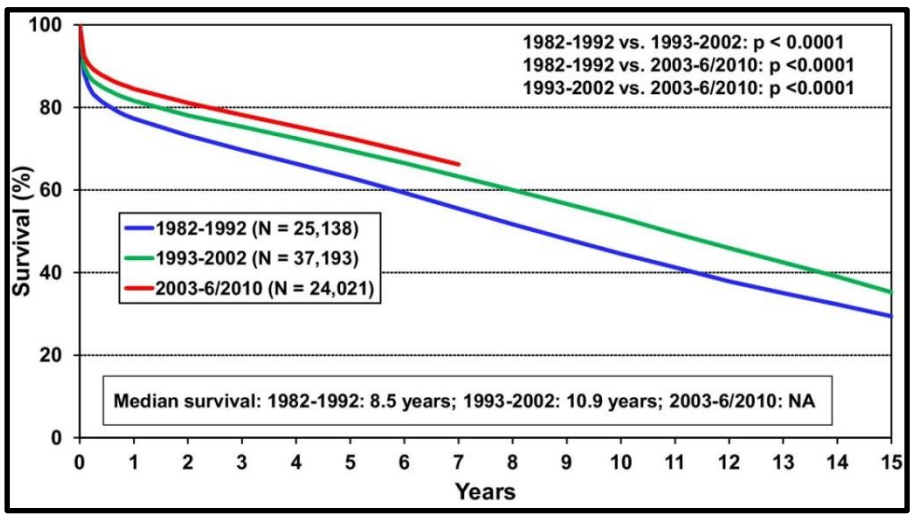


Figure 2.
Survival after HT during different eras. Printed with permission of ISHLT.

Unfortunately, issues related to under- and over-immunosuppression remain common and continue to constitute one of the most important limiting factors for long-term survival. This is clearly reflected in the same report, which concludes that most of the improvement in survival is due to mortality reduction in the first year post-HT and that mortality beyond this time has remained relatively constant over the past 20 years [10]. As this thesis will address this difficult balance, the following parts of this section will provide a brief overview on some of the major points concerning under- and over-immunosuppression following HT.

Transplant immunology

To understand rejection and the mechanisms of action of different immunosuppressants, a look at the initiation of the adaptive immune response is needed. This process begins with alloantigen recognition by a naive T-cell, and is followed by T-cell activation, proliferation and differentiation [11] (**Fig. 3**).

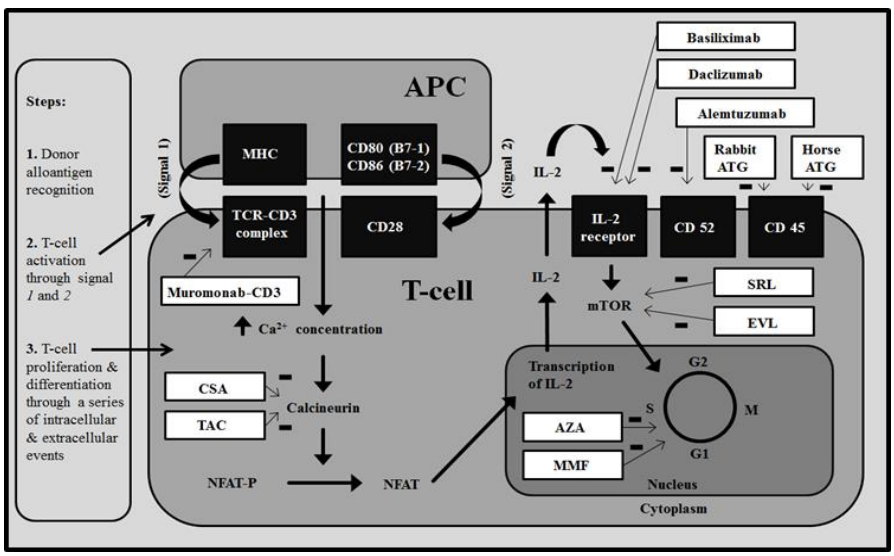


Figure 3.
The initiation of the adaptive immune response and the mechanisms of action of different immunosuppressants on T-cells. Corticosteroids, not seen in the figure, have multiple mechanisms of action on both the innate and adaptive immune response. In lymphocytes however they mainly act by inhibiting the two transcription factors activator protein-1 and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-B). Abbreviations; APC (antigen-presenting cell), ATG (antithymocyte globulin), AZA (azathioprine), CSA (cyclosporine), EVL (everolimus), G1 (cell cycle gap phase 1), G2 (cell cycle gap phase 2), IL-2 (interleukin-2), M (cell cycle mitosis phase), MHC (major histocompatibility complex), MMF (mycophenolate mofetil), mTOR (mammalian target of rapamycin), NFAT (dephosphorylated nuclear factor of activated T-cells), NFAT-P (phosphorylated nuclear factor of activated T-cells), S (cell cycle synthesis phase), SRL (sirolimus), TAC (tacrolimus), TCR (T-cell receptor). "–" indicates inhibition.

Alloantigen recognition depends on antigen-presenting cells (APCs) and their expression of proteins coded for by the major histocompatibility complex (MHC) [11]. APCs can either be dendritic cells, macrophages, certain B- or T-cells, endothelial or epithelial cells. Two MHC molecules are important; class I (A, B, C) and II (DR, DQ, DP) [11]. Whereas class I is located on the surface of most nucleated cells and displays peptides to CD8+ T-cells, class II is often found exclusively on APCs and displays peptides to CD4+ T-cells.

Alloantigen recognition is accomplished through the direct or indirect pathway, in which the presentation of the MHC-peptide combination is mediated through donor or recipient APCs, respectively [12]. After alloantigen recognition, T-cell activation occurs via two signals: i.) the interaction of the T-cell receptor (TCR)-CD3 complex with the MHC on the APC, and ii.) the interaction of co-stimulators such as CD28 on the T-cell with CD80/86 on the APC.

After activation, a series of events lead to proliferation and differentiation of the T-cell [12]. In an important pathway, signal i.) and ii.) cause an increase in cytoplasmic Ca^{2+} concentration, leading to activation of calcineurin and subsequent dephosphorylation of the nuclear factor of activated T-cells (NFAT). NFAT then enters the nucleus and initiates transcription of interleukin-2 (IL-2), which autocrinely leaves the nucleus and binds to the T-cell's IL-2 receptor. This finally activates the mammalian target of rapamycin (mTOR), which regulates transition through the cell cycle, leading to T-cell proliferation and differentiation.

Rejection

Types of rejection

Rejection is classified as hyperacute, acute or chronic [13]. Hyperacute rejection occurs within minutes or hours following donor heart reperfusion. However, due to the use of prospective and virtual crossmatch tests, it is rare nowadays [13]. Acute rejection can be either cellularly or humorally mediated, and consequently termed acute cellular rejection (ACR) or antibody-mediated rejection (AMR). The chronic type of rejection is known as cardiac allograft vasculopathy (CAV). Whereas ACR has been quite well characterized for a long time, AMR and CAV have historically been poorly understood, and it was not until recently that they were recognized as important types of rejection.

Timing and incidence

ACR and AMR typically occur within the first postoperative months [13]. Both may however also appear many years post-HT when CAV otherwise is much more typical [14-15]. The incidences are difficult to estimate due to variations in the definitions of the different types of rejection in different studies. However, the ISHLT recently reported that 19% of all patients included in their registry 2004-2010 had at least one "treatment requiring" acute rejection (i.e. either ACR or AMR) during the first postoperative year, and that 30% vs. 50% of all survivors included 1995-2012 had developed CAV after five vs. 10 years [16]. The definition of "treatment requiring" rejection and the extent of CAV referred to is however not presented in this report.

Pathogenesis, diagnosis and monitoring of ACR

ACR is mainly caused by T-cells directed against the donor heart myocardium, causing inflammation leading to myocyte necrosis and, ultimately, graft failure [17]. EMB still remains the golden standard method for its diagnosis [6,18]. During the procedure, a bioptome is introduced via the right or left internal jugular or femoral vein, whereupon tissue samples of the right ventricular septum are obtained under fluoroscopic and/or echocardiographic guidance (**Fig. 4 a-b**).

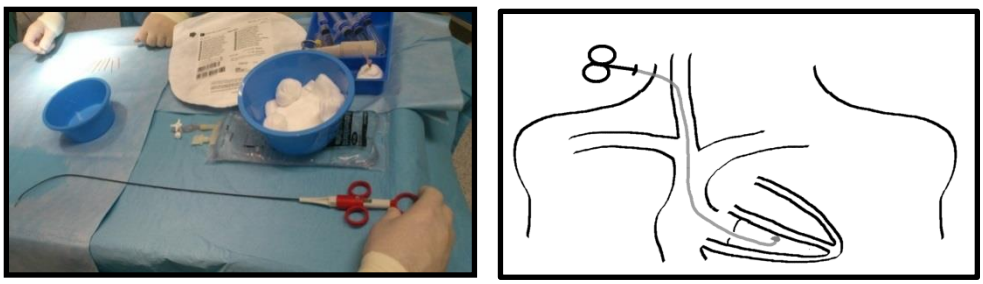


Figure 4 a (left) – b (right).
A bioptome (a) and its course following venous access via the right internal jugular vein (b).

The tissue samples are then graded with respect to severity of ACR according to working formulations (WFs) developed by the ISHLT [19-20] (**Table 1**).

Table 1.
The 1990- & 2004-ISHLT-WF on grading of ACR [19-20].

1990-ISHLT-WF	2004-ISHLT-WF
0 - No ACR	0R - No ACR
1A - Focal, mild ACR	1R - Mild, low-grade ACR
1B - Diffuse, mild ACR	
2 - Focal, moderate ACR	
3A - Multifocal, moderate ACR	2R - Moderate, intermediate ACR
3B - Diffuse, moderate ACR	3R - Severe, high-grade ACR
4 - Severe ACR	

ISHLT (International Society for Heart and Lung Transplantation), WF (working formulation), ACR (Acute cellular rejection). Abbreviated version of original grading system [19-20]. Grading is based on histopathologic findings from endomyocardial biopsies.

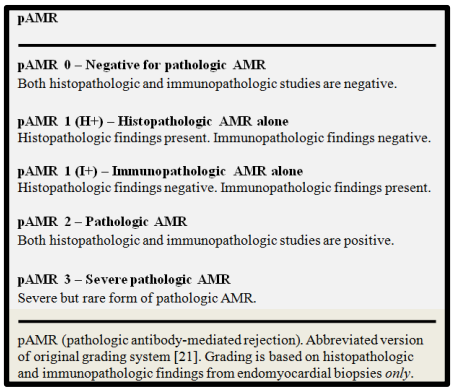
The first WF was introduced in 1990 but then replaced in 2004 to resolve inconsistencies between different centers in the use of the two scales.

Symptoms of ACR include arrhythmia, dyspnea and edema, but also more subtle signs such as fatigue, nausea and fever [13]. It is also well known that ACR can occur without any symptoms at all. This is why practically all HT centers perform pre-planned EMBs on a routine basis. Protocols are highly center-specific but typically include frequent routine EMBs at an early stage, with increasing intervals up to about 12 months post-HT. Whether to continue with routine EMBs at later stages (e.g. > 12 months) remains debated, however, ISHLT recommends continued surveillance beyond this time in "high-risk" patients [13].

Pathogenesis, diagnosis and monitoring of AMR and CAV

AMR is mainly caused by antibodies directed against the donor heart vasculature, causing complement system activation leading to vessel damage and graft failure [17]. The pathogenesis of CAV is not equally well defined. However, it is thought to be caused by a combination of immune and non-immune responses also directed against the donor heart vasculature, leading to a characteristic diffuse and concentric narrowing of the coronary arteries [17]. Agreements on the AMR and CAV nomenclature were first published in 2013 and 2010, respectively [21-22] (**Table 2 a-b**).

Table 2 a (left) – b (right).
The 2013 and 2010 ISHLT agreements on the nomenclature of AMR (a) and CAV (b) [21-22].



pAMR

pAMR 0 – Negative for pathologic AMR
Both histopathologic and immunopathologic studies are negative.

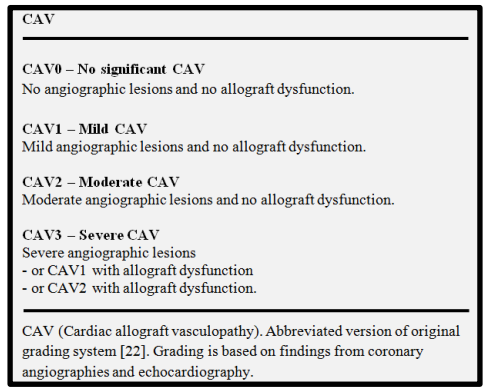
pAMR 1 (H+) – Histopathologic AMR alone
Histopathologic findings present. Immunopathologic findings negative.

pAMR 1 (I+) – Immunopathologic AMR alone
Histopathologic findings negative. Immunopathologic findings present.

pAMR 2 – Pathologic AMR
Both histopathologic and immunopathologic studies are positive.

pAMR 3 – Severe pathologic AMR
Severe but rare form of pathologic AMR.

pAMR (pathologic antibody-mediated rejection). Abbreviated version of original grading system [21]. Grading is based on histopathologic and immunopathologic findings from endomyocardial biopsies *only*.



CAV

CAV0 – No significant CAV
No angiographic lesions and no allograft dysfunction.

CAV1 – Mild CAV
Mild angiographic lesions and no allograft dysfunction.

CAV2 – Moderate CAV
Moderate angiographic lesions and no allograft dysfunction.

CAV3 – Severe CAV
Severe angiographic lesions
- or CAV1 with allograft dysfunction
- or CAV2 with allograft dysfunction.

CAV (Cardiac allograft vasculopathy). Abbreviated version of original grading system [22]. Grading is based on findings from coronary angiographies and echocardiography.

The emerging interest for AMR and CAV has left questions on how screening for these rejection types, which today differs widely between centers, should be conducted. However, in the 2013 agreement on the nomenclature of AMR [21], it is recommended with immunostaining of at least two EMBs during the first month, and thereafter at three, six and 12 months post-HT. The corresponding CAV agreement [22] contains no equivalent recommendation. However, in the latest ISHLT guidelines for the care of heart transplant recipients [13], it is recommended with annual or biannual coronary angiographies until year 3-5, and then less frequently if CAV has been absent. Suspicion of either rejection however calls for additional diagnostic procedures.

Influence on outcome of the different types of rejection

The variation in the timing of the different rejection types is reflected by the fact that ACR and AMR, more often than CAV, contribute to early death [16]. As with incidence estimation, estimating influence on outcome is difficult. However, among all HTs reported to the ISHLT registry 1994-2012, acute rejection (i.e. both ACR and AMR) and CAV were identified to cause 7% and 3% of all mortalities during the first year, respectively, as compared with 2% and 14% of all mortalities year 5-10 post-HT [16].

Issues related to over-immunosuppression

Closely linked to the use of immunosuppressants are a number of medical issues, occurring much more often than in age-matched non-transplanted patients, with potentially devastating influence on outcome. The most important ones include; i.)

infection, ii.) malignancy, and iii.) chronic kidney disease (CKD), the latter related to the use of the calcineurin inhibitors (CNIs) CSA and tacrolimus (TAC) [13]. Recent ISHLT data based on patients reported 1994-2012 [16] correlate well with clinical experience in that infection has a higher relative influence on early mortality than malignancy and CKD, representing the leading death causes month 1-12 vs. year 10-15 at frequencies of 32% vs. 10%, 4% vs. 24%, and 1% vs. 8%, respectively.

With regard to infection, the time post-HT may be divided into three different periods; i.) < one month, ii.) one-six months, and iii.) > six months [23]. Period i.) is dominated by surgical infections, donor-derived infections, and reactivated latent infections. With high immunosuppressive drug levels during period ii.), opportunistic infections may develop, often caused by herpesviruses, pneumocystis jirovecii, toxoplasma gondii and different mycobacteria. During period iii.), when the drug levels are lower, common respiratory and urinary tract infections tend to dominate. Unfortunately, clinical and radiological findings may be nonspecific why diagnosis often is challenging [23].

Of the malignancies, non-melanoma skin cancer, post-transplant lymphoproliferative disorder, anogenital cancer and Kaposi's sarcoma are often the most common [24]. According to ISHLT data [16], 14% and 28% of all patients reported 1994-2012 were affected by any cancer within the fifth and tenth postoperative year, respectively [16]. Among the same patients, during the same time period, the proportions of patients requiring dialysis or renal transplantation were also found to be high (3% and 1% within five years as well as 6% and 4% within 10 years) [16].

Induction and maintenance immunosuppression

The induction and maintenance (temporary perioperative and life-long, respectively) immunosuppressive drugs and therapies in HT are described below.

Induction immunosuppression - overview

Historically and contemporary tested and/or used induction immunosuppressants, their effects and side effects, are shown in **Table 3**. The mechanisms of action can be seen in **Fig. 3**.

Table 3.
Historically and contemporary tested and/or used induction immunosuppressants.

Drug class / drug	Effects	Important side effects (not infection and malignancy)
Antithymocyte globulin Rabbit antithymocyte globulin ● Thymoglobulin® (Genzyme) ● ATG-Fresenius® (Fresenius) Horse antithymocyte globulin ● ATGAM® (Pfizer)	Rapid depletion of T- and B-cells	Thrombocytopenia, leukopenia, cytokine release syndrome, serum sickness
Interleukin-2 receptor antagonists Basiliximab ● Simulect® (Novartis) Daclizumab ● Zenapax® (Roche) ● Zinbryta® (Biogen Sweden)	Prevention of proliferation and differentiation of T-cells	Relatively well tolerated
Anti-CD3 antibodies Muromonab-CD3 ● Orthoclone OKT3® (Janssen-Cilag)	Rapid depletion of T-cells	Cytokine release syndrome, serum sickness
Anti-CD52 antibodies Alemtuzumab ●Campath® (Genzyme) ●Lemtrada® (Sanofi)	Rapid depletion of T- and B-cells	Poorly studied, persistent leukopenia

Note: Zenapax® and Orthoclone OKT3® were withdrawn 2009 due to decreased demand. Campath® was withdrawn 2012 whereas Lemtrada® was introduced 2013. Lemtrada® is however not approved for use in heart transplantation. The same applies to Zinbryta®, introduced 2016.

Induction immunosuppression – current use and trends

According to the ISHLT registry [16], the use of any induction immunosuppressant remained stable during the past decade (52% of all patients 2002 vs. 47% 2012). In recent years, the preferred type of drugs have been antithymocyte globulins (ATGs) or IL-2 receptor antagonists, used in 27% and 21% vs. 19% and 28% of all HTs 2002 vs. 2012, respectively [16].

Induction immunosuppression – controversies

The fact that only around half of all HT recipients worldwide receive induction reflects the controversies regarding its use. Benefits may include earlier reduction of corticosteroids (CSs) [25] and delayed initiation of CNIs [26-27], without a higher rejection risk. Little is however known regarding the efficacy and safety of the drugs used. In particular, their long-term adverse effects are incompletely understood, and it is feared that they entail an increased risk of malignancy [13].

A Cochrane review with a meta-analysis of 22 randomized trials [28] recently addressed the use of induction therapy in HT patients. Major outcomes studied were; mortality, ACR, CAV, infection, malignancy and renal function. In a sub analysis comparing "induction vs. no induction" (five trials of which none compared ATG vs. no induction), there were less ACRs with the use of induction. This was however not seen using a "random-effects-model". Moreover, significant differences could

not be found for any of the other outcomes evaluated. A large, more recent, retrospective study [29] also failed to detect any survival benefits with induction over no induction.

Induction immunosuppression – ATGs and IL-2-receptor antagonists

ATGs are polyclonal depleting antibodies as opposed to IL-2 receptor antagonists which are monoclonal non-depleting equivalents. ATGs bind to several antigens on both T- and B-cells causing T- and B-cell depletion. IL-2 receptor antagonists, in contrast, act by binding the IL-2 receptor on T-cells and thus specifically inhibit the proliferation and differentiation of T-cells (**Fig. 3**) [30-31].

Although together used in almost half of all HTs worldwide, ATGs and IL-2 receptor antagonists have been poorly studied. The few randomized trials available typically include around 50 patients and have short follow-up times [30-31]. The lack of data on safety and efficacy in HT patients is an important reason to why Simulect®, the only currently available IL-2 receptor antagonist of which clinical experience in HT exist, officially only is indicated in kidney but not heart transplant patients [32-33].

Based on the results of eight retrospective studies [29,34-40] and three randomized trials [41-46] it appears as if both types of drugs may be beneficial in preventing ACR, but that neither seem to influence survival. Some but not all data however indicate that patients given ATG have better outcome than those given IL-2 receptor antagonists [29,39-40], and that IL-2 receptor antagonists also are less efficient than ATG in preventing ACR [37].

Induction immunosuppression – Anti-CD3 antibodies

Anti-CD3 antibodies are monoclonal antibodies that bind to CD3 on T-cells, thereby causing rapid depletion of already existing T-cells (**Fig. 3**) [30-31]. As seen in **Table 3**, Orthoclone OKT3® was withdrawn in 2009 due to decreased demands. It is not likely to return as earlier studies on its safety and efficacy have been discouraging [47].

Induction immunosuppression – Anti-CD52 antibodies

Anti-CD52 antibodies are also monoclonal antibodies, but bind to CD52 on both T- and B-cells, thus depleting both existing T- and B-cells (**Fig. 3**) [30-31]. Early data from a small retrospective study [48] and a small randomized trial [49] were promising, indicating similar outcome but fewer rejections with than without it. These initial results were then confirmed in a larger case control series [50-54]. Data are however still considered too insufficient to recommend its routine use [55].

Maintenance immunosuppression – overview

Maintenance immunosuppression after HT usually consists of a triple combination of; i.) CSs, ii.) a CNI (CSA or TAC), and iii.) an antimetabolite (azathioprine [AZA] or mycophenolate mofetil [MMF]). Everolimus (EVL) or sirolimus (SRL), both mTOR inhibitors, are sometimes also used. Typically, CSs are gradually tapered to a very low dose, or sometimes completely withdrawn, by the first year. CNI-doses are tapered in a similar way, although guided by regular drug concentration measurements. The antimetabolites are usually kept at stable doses.

The effects and side effects of the above drug classes / drugs, are shown in **Table 4**. The mechanisms of action can be seen in **Fig. 3**.

Table 4.
Maintenance immunosuppressants after HT.

Drug class / drug	Effects	Important side effects (not infection and malignancy)
Corticosteroids		
Prednisolone / Prednisone	Wide, on both the innate and adaptive immune response	Diabetes, osteoporosis, anxiety, depression, amenorrhea, cataract, glaucoma, Cushing's syndrome
Calcineurin inhibitors		
Tacrolimus	Prevention of proliferation and differentiation of T-cells	Drug-drug interactions, nephrotoxicity, neurotoxicity, hypertension, dyslipidemia, diabetes
Cyclosporine		Drug-drug interactions, nephrotoxicity, neurotoxicity, hypertension, dyslipidemia, gingival hyperplasia, hirsutism
Antimetabolites		
Mycophenolate mofetil Azathioprine	Prevention of proliferation and differentiation of T- and B-cells	Leukopenia, gastrointestinal problems Pancytopenia, hepatitis, pancreatitis
Mammalian target of rapamycin inhibitors		
Everolimus Sirolimus	Prevention of proliferation and differentiation of T- and B-cells	Drug-drug interactions, dyslipidemia, pancytopenia, delayed wound healing, oral ulcers, pericardial and pleural effusions

Maintenance immunosuppression – CSs

CSs form an important component of the maintenance therapy and exhibit multiple mechanisms of action that affect both the innate and adaptive immune response. In lymphocytes however they primarily act through the inhibition of the two transcription factors activator protein-1 and nuclear factor kappa-light-chain-enhancer of activated B-cells [56].

Maintenance immunosuppression – CNIs and antimetabolites

Whereas the CNIs (CSA and TAC) inhibit the enzyme calcineurin in T-cells and thereby prevent the proliferation and differentiation T-cells, the antimetabolites (AZA and MMF) inhibit the cell cycle in both T- and B-cells, thereby exerting a slightly broader effect (**Fig. 3**) [56]. According to the ISHLT registry, the use of CNIs and antimetabolites remained stable during the past decade (98% and 88% of all patients 2000 as well as 94% and 89% of all patients 2012, at one year post-HT, respectively) [16]. CSA and AZA have however largely been replaced by the newer TAC and MMF (of patients transplanted 2012 used at one year post-HT in 13% vs. 81% for CSA vs. TAC and 3% vs. 85% for AZA vs. MMF). These trends are likely due to reports indicating benefits with TAC and MMF. With regard to the CNIs, some of the benefits with TAC over CSA were recently reported in an in-depth meta-analysis of 10 randomized trials [57]. The results indicated that TAC was superior to CSA in terms of hypertension, hyperlipidemia, hirsutism and gingival hyperplasia, but there was no difference in mortality, severe ACR, CAV, infection, malignancy, renal function or diabetes mellitus. However, when separately analyzing oil-based and microemulsion CSA, TAC was also superior to microemulsion CSA with regard to mortality and ACR prevention.

Maintenance immunosuppression – mTOR inhibitors

The mTOR inhibitors EVL and SRL act by inhibiting the enzyme mTOR found in T- and B-cells, thereby preventing proliferation and differentiation of T- and B-cells (**Fig. 3**) [56]. According to the ISHLT registry, the use of mTOR inhibitors has increased (3% and 13% of patients at one year post-HT in 2000 and 2012, respectively). As described below, they are currently used in patients with, or at increased risk of, CKD and CAV. Their use is however sometimes limited by their adverse effects [58].

CNI-minimization

CNI-minimization is desirable, especially due to their nephrotoxic side effects. Several strategies have been evaluated of which some have proven successful, notably the use of induction therapy to delay CNI-initiation, as shown in a retrospective [26] and prospective [27] study, and the use of MMF over AZA to reduce CNI-doses, as shown in several studies (notably [59] and [60]). As both strategies indicated beneficial effects on renal function without increased rejection risk, induction therapy and MMF over AZA seem advisable in patients with CKD.

CNI-minimization with mTOR inhibitors has, moreover, also been studied. Initial randomized trials [61-62] indicated that EVL and SRL actually potentiate the nephrotoxic side effects of CNIs unless CNI-doses are reduced. Several studies have

subsequently studied the introduction of an mTOR inhibitor together with a CNI-reduction [63-82] or withdrawal [77-109]. Altogether it appears as if both strategies may have beneficial effects on renal function. Several of the studies also compared reduction vs. withdrawal [77-82, 108-109], but most were unable to find any differences in renal function and acute rejection. However, regardless if a strategy based on reduction or withdrawal is attempted, early rather than late minimization seems favorable [66-67,76,78,83].

The largest randomized trials on CNI-reduction and withdrawal are NOCTET [66-67,76] and SCHEDULE [103,106]. Patients with moderate CKD made up the study group of both trials. In NOCTET, a CNI-reduction and concurrent EVL-introduction was made ≥ one year post-HT, whereas in SCHEDULE EVL was introduced within five days and the CNI withdrawn between week 7-11. In both studies, glomerular filtration rate (GFR) was better in the study than the control group maintained on standard CNI-doses. This difference also persisted after five and three years. In addition, ACR rates were similar, except for during the first year of SCHEDULE.

CS-minimization

Due to the well-known side effects of long-term treatment with CSs, minimizing their use is also very important. Early CS-withdrawal, late CS-withdrawal, and CS-avoidance may all be possible in suitable patients. In fact, in a recent review [110], the authors concluded that CS-withdrawal is safe in 50-80% of all patients, with late being better than early, and that CS-avoidance should be advisable and mandatory in pediatric and elderly patients, those with severe diabetes mellitus, familial metabolic disorders, osteoporosis and recurrent infections.

Treatment of ACR and AMR

Treatment of ACR and AMR differs between centers, but recommendations can be found in the 2010 ISHLT guidelines for the care of heart transplant recipients [13]. An abbreviated version of the recommendations on ACR is seen in **Table 5**.

Table 5.
Recommendations on the treatment of ACR [13].

ACR grade (2004-ISHLT-WF)	Recommended treatment
Asymptomatic ACR grade 1R	Usually no treatment required
Asymptomatic ACR grade 2R	High-dose oral CSs for three to five days – or – High-dose intravenous CSs for three days
Asymptomatic ACR grade 3R	High-dose intravenous CSs for three days – and – Addition of ATG in case of evidence of graft dysfunction or absence of histologic resolution
Symptomatic ACR (grade 1R-3R)	High-dose intravenous CSs for three days – and – Addition of ATG in case of hemodynamic compromise or no improvement after 12-24h

ISHLT (International Society for Heart and Lung Transplantation), ACR (Acute cellular rejection), WF (Working formulation), CSs (Corticosteroids), ATG (antithymocyte globulin). Abbreviated version of the original guidelines [13].

As the above mentioned guidelines were published before the AMR nomenclature agreement, a protocol based on AMR grades similar to that of ACR does not yet exist. However, in the 2010 ISHLT guidelines, high-dose intravenous CSs, ATG, anti-CD3 antibodies, plasmapheresis and immunapheresis are all mentioned as alternatives [13], as is the anti-CD20 antibody Rituximab which has shown useful earlier [111].

Prevention of CAV

In contrast to ACR and AMR, CAV is difficult to treat once already established. Due to its diffuse nature, both percutaneous and surgical revascularization is of quite limited value. Identifying ways of preventing CAV is therefore important. Routine screening with coronary or computed tomography (CT) angiography may be one approach. Randomized trials have also indicated lower CAV risk after de-novo treatment with statins [112-115], the calcium channel blocker diltiazem [116-117], TAC instead of CSA [118], MMF instead of AZA [119], and with the mTOR inhibitors EVL [61] and SRL [62] rather than AZA. More recently in SCHEDULE [103,106], CAV was better prevented in those given EVL than CSA.

HT in Lund – from past to present

HTs have been performed at Skåne University Hospital in Lund (SUS-Lund) since 1988. The first one was performed by the cardiac surgeon Jan-Otto Solem, only four years following the first HT ever in Sweden at Sahlgrenska University Hospital in Gothenburg in 1984 (**Fig 5.**).



Figure 5 a (left) – c (right).
The cardiac surgeon Jan-Otto Solem (a) and SUS-Lund in 1988 (b). Figure (c) shows the second patient to undergo HT in Lund - Leif Redestig - at the time of his 28th yearly check-up. Mr. Redestig today awaits his 29th yearly check-up and, although 66 years old, he still works near full-time. At the time of HT he was 37 years old and a father of three small children. Affected by severe heart failure with repeated ventricular storms, he remembers the time prior to the HT as shattering. Out of coincidence, he came across an article about HT in a popular scientific magazine he happened to have next to his bed. He understood that the concept of HT was new at that time, and was therefore very surprised when he was offered to undergo the procedure. The decision was easy as he knew his prognosis was very poor. The new heart arrived after only three days. He remembers Jan-Otto Solem later telling him "his old heart was the biggest one he had ever seen". Printed with signed permission of Mr. Redestig.

Beginning with the first patient, the cardiologists Stig Persson followed by Björn Kornhall established an extensive follow-up program which still persists today. As part of this program, HT patients at SUS-Lund have come for regular check-ups including; i) visits to physicians, nurses, physiotherapists, occupational therapists, counselors and dieticians, ii) blood test samplings and iii) diagnostic investigations including EMB and hemodynamic measurement via right heart catheterization, electrocardiography, echocardiography, heart-lung X-ray, exercise test, myocardial scintigraphy, coronary or CT angiography, and measurement of GFR and bone density with the iohexol clearance method and dual X-ray absorptiometry, respectively (**Fig. 6**).

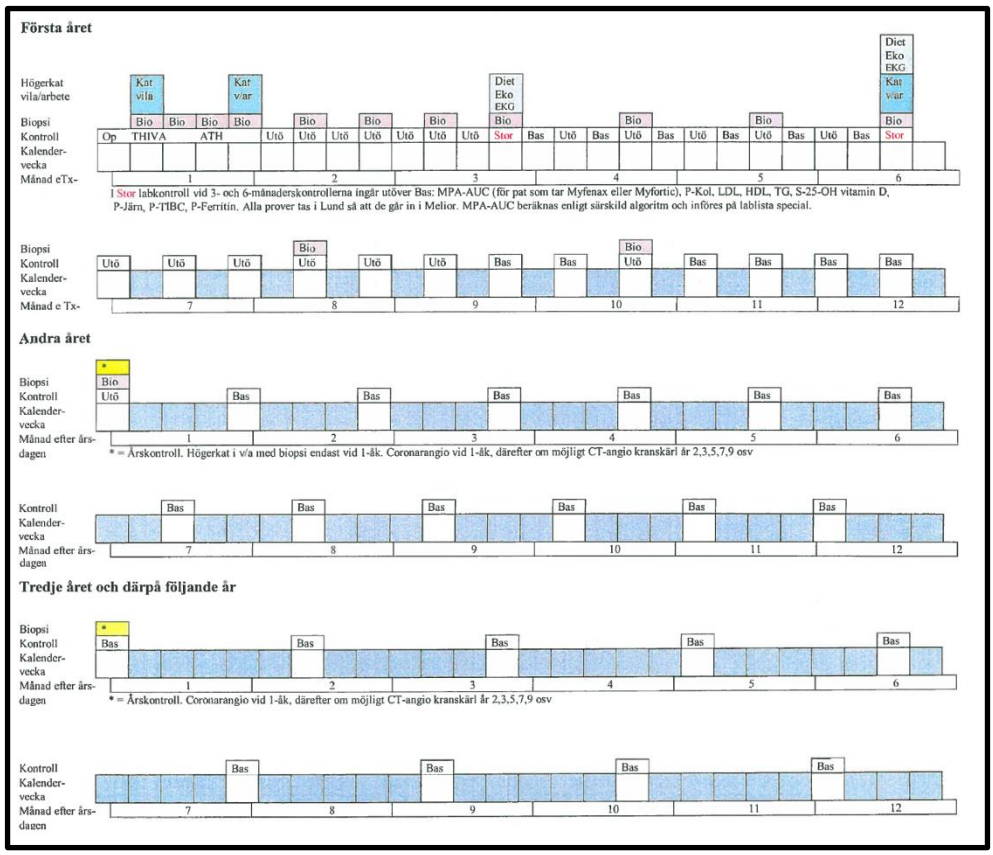


Figure 6.
A schematic overview of the extensive follow-up program in Lund, intended for the patients. Designed by Dr. Björn Kornhall. In Swedish.

The extensive follow-up program has not only been valuable for the individual patients that have undergone HT, but has also led to a steadily growing clinical experience on management of HT patients at SUS-Lund. This is also reflected by the recent five-year renewal of SUS-Lund´s permission to serve as one of two national referral centers for HT in Sweden - the other being previously mentioned Sahlgrenska University Hospital in Gothenburg [120].

Several clinics at SUS-Lund contribute with their specific expertise in the daily management of the HT patients and together make up the HT team. However, most follow-up is conducted via the Hemodynamic Lab at the cardiology department, currently coordinated by Dr. Göran Rådegran (**Fig. 7**).

Figure 7 a (left) – b (right).
From left to right - senior cardiologists Johan Holm, Øyvind Reitan, Björn Kornhall and Göran Rådegran inside the Hemodynamic Lab at the cardiology department in Lund. Dr. Stig Persson is seen to the right.

Although the clinical experience has grown, a retrospective collection and subsequent evaluation of data obtained throughout the years has not been made previously. Therefore, to gain knowledge from the past for future use, Dr. Rådegran initiated the Lund Heart Transplantation Research Register (LHTRR) in 2014. This database is based on data collection commenced in 2010, intended for the present and two other theses. It is planned to continue to grow and to serve as platform for various projects to be undertaken ahead.

Aims

The general aim of the present thesis was to gain insight into the difficult balance between under- and over-immunosuppression after HT, and to contribute with the first data to LHTRR for future projects. Two of the most clinically relevant issues, namely ACR and CKD, were the focus of the first three papers. The final paper aimed to evaluate the safety and efficacy of switching to generic immunosuppressants – a debated clinical issue closely related to the difficult immunosuppressive balance.

Specific aims of each paper were:

- To study the incidence, predictors and outcome of:
  - Early ACR, defined as ACR the first year after HT (Paper **I**)
  - Late ACR, defined as ACR later than one year after HT (Paper **II**)
  - CKD after HT (Paper **III**)

- To investigate the safety and efficacy of switching stable HT patients from CellCept® to Myfenax Teva® and/or from Prograf® to Tacrolimus Sandoz® (MMF and TAC, respectively) (Paper **IV**).

In Paper **III**, an additional aim was to evaluate the accuracy of two guideline recommended GFR estimating equations (CKD-EPI and Schwartz formulae) among HT patients.

Methods

Paper **I-III**

Study population

Paper **I-III** were based on all 215 HT patients followed at SUS-Lund 1988-2010. Among the 215 patients, 219 HTs had been performed. Of these, 214 (98%) were first-time HTs and five (2%) were re-HTs (three within seven days, one 175 days and one 18 years after HT). All HTs except for a pediatric first-time HT performed abroad in 2006 had been performed at SUS-Lund. One patient who underwent re-HT in 2002 had originally been transplanted abroad in 1984.

The mean number of HTs performed per year over the course of the study period 1988-2010 was 9.5 (standard deviation 4.3, min 0, max 17). **Fig. 8** illustrates the number of HTs each year, stratified by the presence or absence of prior ventricular assist device and age.

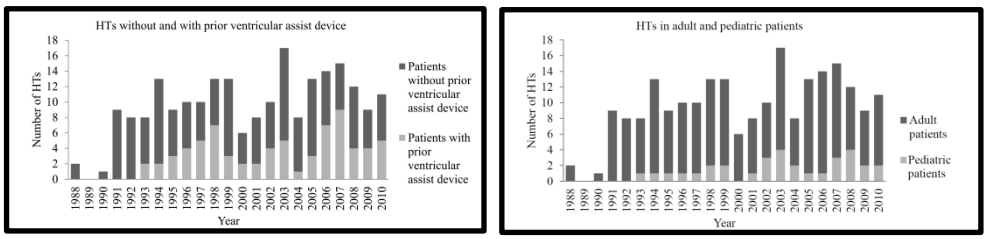


Figure 8 a (left) – b (right).
The number of HTs each year at SUS-Lund 1988-2010 in (a) patients without and with prior ventricular assist device and (b) adult and pediatric patients below 18 years of age.

In paper **II**, 18 of the 215 patients could not be included due to death within the first year. Another 14 patients also had to be excluded as the performance and analysis of EMBs later than one year post-HT had been made at other centers. In paper **III**, 81 of the 215 patients had to be excluded as the long-term follow-up of renal function and associated parameters also had been managed elsewhere. Study

population characteristics of the patients included in each paper are shown in Table **6-7**.

Table 6.
Study population characteristics of patients included in Paper **II** vs Paper **I** (representing the entire cohort). Paper **II** included four re-HTs.

Characteristics	Entire cohort (Paper **I**)(219 HTs)			Paper **II** (186 HTs)			p
	Median	n	%	Median	n	%	
Age of recipient at HT (years) =	**51.0**	219		**51.0**	186		0.94
Pediatric recipients (below 18 years of age)		32	**14.6**		23	**12.4**	0.61
Age of donor at HT (years) =	**44.0**	219		**44.0**	186		0.99
Pediatric donors (below 18 years of age)		17	**7.8**		13	**7.0**	0.92
Difference in age between recipient and donor (years +/-)	**9.0**	219		**9.0**	186		0.83
0-11		127	**58.0**		111	**59.7**	0.81
12-22		49	**22.4**		40	**21.5**	0.93
23-33		32	**14.6**		25	**13.4**	0.85
34-45		11	**5.0**		10	**5.3**	0.95
Gender of recipient		219			186		
Female		70	**32.0**		60	**32.2**	0.97
Gender of donor		219			186		
Female		82	**37.4**		72	**38.7**	0.87
Sex-matching between recipient and donor		219			186		
Sex-mismatched		58	**26.5**		48	**25.8**	0.97
ABO-matching between recipient and donor		219			186		
ABO-identical		184	**84.0**		156	**83.9**	0.93
ABO-compatible		32	**14.6**		28	**15.1**	0.71
ABO-incompatible		3	**1.4**		2	**1.1**	0.85
CMV-constellation		204			176		
Donor + / Recipient +		107	**52.5**		90	**51.1**	0.88
Donor - / Recipient -		20	**9.8**		20	**11.3**	0.74
Donor + / Recipient -		28	**13.7**		25	**14.2**	0.99
Donor - / Recipient +		49	**24.0**		41	**23.2**	0.96
First-year ACR ≥ grade 2		219			186		
Yes		114	**52.1**		99	**53.2**	0.89
VAD prior to HT		219			186		
Yes		72	**32.9**		62	**33.3**	0.99
Recipient diagnosis		219			186		
Dilated cardiomyopathy		113	**51.6**		100	**53.8**	0.74
Ischemic cardiomyopathy		59	**26.9**		48	**25.8**	0.89
Other heart disease		47	**21.4**		38	**20.4**	0.90
Waiting time (days)	**80.0**	219		**79.0**	186		0.81
0-322		192	**87.7**		159	**85.4**	0.62
323-644		19	**8.7**		19	**10.2**	0.72
645-966		6	**2.7**		6	**3.2**	1.00
967-1289		2	**0.9**		2	**0.1**	0.73
Ischemic time (minutes)	**194.5**	216		**194.0**	184		0.92
46-126		48	**22.2**		43	**23.4**	0.88
127-207		84	**38.9**		71	**38.6**	0.97
208-288		78	**36.1**		66	**35.9**	0.96
289-369		6	**2.8**		4	**2.2**	0.89
Induction immunosuppression		214			183		
Antithymocyte globulin		201	**93.9**		173	**94.5**	0.97
Daclizumab		8	**3.7**		7	**3.8**	0.83
No induction		5	**2.3**		3	**1.6**	0.89
Maintenance immonosuppression in patients alive at discharge		204			183		
Cyclosporine + Azathioprine + Corticosteroids		99	**48.5**		87	**47.5**	0.93
Cyclosporine + MMF/MPA + Corticosteroids		80	**39.2**		75	**41.0**	0.80
Tacrolimus + MMF/MPA + Corticosteroids		13	**6.4**		10	**5.5**	0.87
Other combinations		12	**5.9**		11	**6.0**	0.87

Abbreviations: Heart transplantation (HT), n (Number of heart transplantations in each group), CMV (Cytomegalovirus), ACR (Acute cellular rejection), VAD (ventricular assist device), MMF/MPA (Mycophenolate mofetil/ acid). p – comparison of bold values.

Table 7.
Study population characteristics of patients included in Paper **III** vs Paper **I** (representing the entire cohort). Paper **III** included three re-HTs. One of the patients in paper **III** underwent a simultaneous heart and kidney transplant.

Characteristics	Entire cohort (Paper **I**)(219 HTs)				Paper **III** (137 HTs)				p
	Mean	SD ±	n	%	Mean	SD ±	n	%	
Age of recipient at HT (years) =	**44.6**	17.2	219		**44.4**	18.3	137		0.88
Pediatric recipients (below 18 years of age)			32	**14.6**			24	**17.5**	0.56
Age of donor at HT (years) =	**40.1**	15.7	219		**39.2**	16.7	137		0.60
Pediatric donors (below 18 years of age)			17	**7.8**			14	**10.2**	0.54
Difference in age between recipient and donor (years +/-)	**12.4**	10.8	219		**12.9**	11.4	137		0.64
0-11			127	**58.0**			77	**56.2**	0.83
12-22			49	**22.4**			31	**22.6**	0.94
23-33			32	**14.6**			19	**13.9**	0.97
34-45			11	**5.0**			10	**7.3**	0.51
Gender of recipient			219				137		
Female			70	**32.0**			41	**30.0**	0.78
Gender of donor			219				137		
Female			82	**37.4**			54	**39.4**	0.79
Sex-matching between recipient and donor			219				137		
Sex-mismatched			58	**26.5**			41	**29.9**	0.56
ABO-matching between recipient and donor			219				137		
ABO-identical			184	**84.0**			113	**82.5**	0.82
ABO-compatible			32	**14.6**			22	**16.1**	0.83
ABO-incompatible			3	**1.4**			2	**1.5**	0.70
CMV-constellation			204				131		
Donor + / Recipient +			107	**52.5**			72	**55.0**	0.74
Donor - / Recipient -			20	**9.8**			15	**11.5**	0.77
Donor + / Recipient -			28	**13.7**			16	**12.2**	0.82
Donor - / Recipient +			49	**24.0**			28	**21.4**	0.67
Recipient diagnosis			219				137		
Dilated cardiomyopathy			113	**51.6**			74	**54.0**	0.74
Ischemic cardiomyopathy			59	**26.9**			32	**23.4**	0.53
Other heart disease			47	**21.4**			31	**22.6**	0.90
Waiting time (days)	**150.4**	200.7	219		**144.1**	181.5	137		0.77
0-322			192	**87.7**			120	**87.6**	0.89
323-644			19	**8.7**			14	**10.2**	0.76
645-966			6	**2.7**			2	**1.5**	0.67
967-1289			2	**0.9**			1	**0.7**	0.68
Ischemic time (minutes)	**185.0**	63.5	216		**183.2**	61.9	135		0.80
46-126			48	**22.2**			30	**22.2**	0.90
127-207			84	**38.9**			56	**41.5**	0.71
208-288			78	**36.1**			45	**33.3**	0.68
289-369			6	**2.8**			4	**3.0**	0.82
Induction immunosuppression			214				134		
Antithymocyte globulin			201	**93.9**			124	**92.5**	0.78
Daclizumab			8	**3.7**			7	**5.2**	0.69
No induction			5	**2.3**			3	**2.2**	0.76
Maintenance immonosuppression in patients alive at discharge			204				129		
Cyclosporine + Azathioprine + Corticosteroids			99	**48.5**			56	**43.4**	0.42
Cyclosporine + MMF/MPA + Corticosteroids			80	**39.2**			50	**38.8**	0.52
Tacrolimus + MMF/MPA + Corticosteroids			13	**6.4**			12	**9.3**	0.44
Other combinations			12	**5.9**			11	**8.5**	0.48
Treatment with Tacrolimus at year 1									
Yes			-	-			91	-	-
Treatment with Cyclosporine at year 1									
Yes			-	-			22	-	-
Treatment with ACEI or ARB at year 1			-				120		
Yes			-	-			40	**33.3**	-
Proteinuria at year 1			-				79		
Yes			-	-			13	**16.5**	-
First-year ACR ≥ grade 3A/3B			219				129		
Yes			78	**35.6**			51	**39.5**	0.54

Abbreviations: HT (Heart transplantation), SD (Standard deviation), n (Number of heart transplantations in each group), CMV (Cytomegalovirus), MMF/MPA (Mycophenolate mofetil/ acid), ACEI (Angiotensin converting enzyme inhibitor), ARB (Angiotensin receptor blocker), ACR (Acute cellular rejection). p – comparison of bold values.

Immunosuppression

The immunosuppressants used for induction and maintenance therapy can be seen in **Table 6-7**. **Fig. 9** illustrates the immunosuppression according to different eras (1988-2010, 1988-1999 and 2000-2010) for the entire patient cohort.

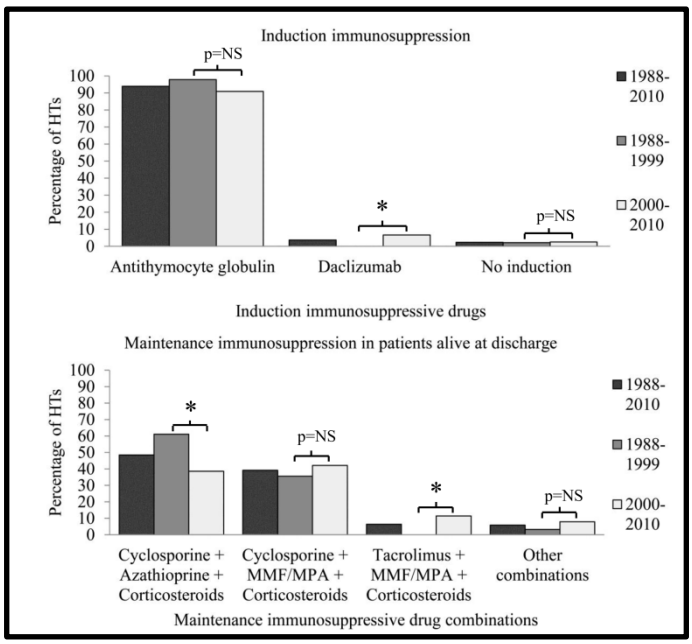


Figure 9.
Immunosuppression according to different eras for the entire patient cohort. NS = not significant. * Indicates statistical significance ($p<0.05$).

The vast majority of all patients received induction with ATG, irrespective of era. Over the course of the entire study period 1988-2010, the most common maintenance therapy consisted of CSA + AZA + CS. However, over the years, CSA and AZA were largely replaced by TAC and MMF, respectively.

Following discharge, CSA and TAC trough (C0) levels had typically been monitored once a week, months 0-6; and every other week, months 6-12. C0 levels had generally been targeted, respectively, to; 250-300 and 12-15 µg/l, months 0-2; 200-275 and 10-14 µg/l, months 2-4; 150-225 and 8-12 µg/l, months 4-6; 100-175 and 6-10 µg/l, months 6-12; as well as 80-130 and 5-8 µg/l, after the first year. Adult patients had generally received a daily CS-dose of 20 mg, months 0-2; 15 mg, months 2-4; 7.5-12.5 mg, months 4-6; 5.0-7.5 mg, months 6-12; as well as 2.5-5.0 mg, beyond year 1. In contrast, pediatric patients had been given 10 mg/m^2/day,

months 0-2; 7.5 mg/m^2/day, months 2-3; 5.0 mg/m^2/day, months 3-4; 2.5 mg/m^2/day months 4-5; as well as 2.5 mg/m^2 every second day, months 5-6. Thereafter, children without severe rejection had usually been weaned off CSs.

EMBs

The majority of the EMBs had been performed at the Hemodynamic Lab, the Section for Heart Failure and Valvular Disease, at SUS-Lund, and analyzed at the Department of Pathology, also at SUS-Lund. EMBs were characterized as either routine or additional clinically indicated (ACI) EMBs. Routine EMBs had been performed on a routine basis on 14 occasions during the first year (week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40 and 52 post-HT). Beyond year 1, routine EMBs had usually been performed annually or biennially. In patients free from rejection for several consecutive years, routine EMBs had however sometimes been phased out completely. A more restrictive protocol on routine EMBs beyond year 1 had also been adopted over time due to accumulated experience of low yields of ACR. ACI EMBs had been obtained following clinical symptoms or previous episodes of ACR, regardless of time post-HT. In general, the same routine and ACI EMB schedule had been adopted in pediatric and adult patients. Four to five myocardial specimens were typically obtained during each EMB. The EMBs had been graded according to the 1990- and 2004-ISHLT-WF [19-20] (**Table 1**) in parallel from 2005 and onwards. However, as most of the EMBs solely had been graded according to the original 1990-ISHLT-WF, the 1990-ISHLT-WF was adhered to during analysis.

2990 (2635 routine, 355 ACI) early and 815 (383 routine, 432 ACI) late EMBs were available for inclusion. The numbers of early and late routine and ACI EMBs for each year of, and each week/year after, HT, are shown in **Fig. 10**.

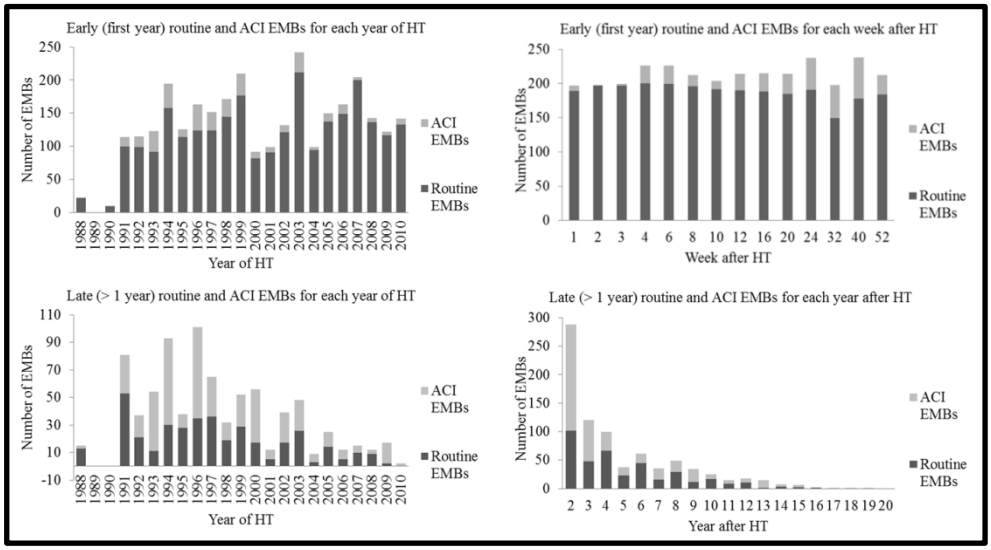


Figure 10.
The numbers of early and late routine and ACI EMBs for each year of, and each week/year after, HT.

GFR measurements

As part of the follow-up program previously described, patients followed at SUS-Lund had come for yearly measurements of GFR based on the plasma clearance of the filtration marker iohexol [121-122]. These measurements had also been performed prior to HT. As iohexol almost exclusively is eliminated through the kidneys [121-122], its use enables accurate determination of GFR as compared to GFR estimating equations [123].

Before the measurements, estimated GFR had been calculated to obtain optimal sampling schemes. Patients had then been administered a dose adjusted solution of iohexol (Omnipaque®, GE Healthcare), whereupon single or multiple plasma sampling had been performed depending on patient morphometrics – usually after four hours and, in those with atypical body constitution, another sample at least one hour thereafter. In patients with poor renal function, plasma sampling had sometimes been delayed up to 72 hours. High-performance liquid chromatography had then been used to obtain iohexol concentrations.

Data collection

Data from EMBs and iohexol clearance measurements, together with data on potential predictors, survival and additional pre- and post-HT variables of interest,

were retrospectively collected from medical records. End of follow-up was 30th June 2012.

Pre-analysis preparations

In paper **I-II**, the intention was to focus on clinically relevant ACRs, such as ACRs severe enough to require specific rejection treatment. The ISHLT guidelines recommend treatment of asymptomatic ACR ≥ grade 2R (≥ original 3A) and symptomatic ACR irrespective of EMB grade [13]. This approach is similar to the one used at SUS-Lund during the time period studied. Treatment had however occasionally also been given to asymptomatic patients with early postoperative ACR of grade 1R (original 1A/1B or 2) or three consecutive ACRs of grade 1R without resolution. Most of the comparative analyses in paper **I-II** therefore focused on ACRs ≥ original grade 2 rather than 3A.

In paper **III**, the amount of data available for analysis was increased by applying GFR estimating equations when iohexol clearance measurements were lacking, but when S-creatinine values were available. Based on the latest guidelines from KDIGO (Kidney Disease: Improving Global Outcomes) [122], the CKD-EPI [125] and Schwartz [126] formulae were used in adults and children, respectively (**Table 8**). Iohexol clearance measurements nonetheless constituted the primary source of all the GFR data, representing 90.4% of all 923 data points in adults and 88.1% of all 101 data points in children.

Table 8.
The CKD-EPI and Schwartz formulae.

- **CKD-EPI**

$$GFR\ [mL/min/1.73m^2] = 141 * \min(S\text{-}cr\ [mg/dL]) / \kappa, 1)^{\alpha} * \max(S\text{-}cr\ [mg/dL]) / \kappa, 1)^{-1.209} * 0.993^{Age} * 1.018\ [if\ female] * 1.159\ [if\ black]$$

- **Schwartz ("Bedside Schwartz")**

$$GFR\ [mL/min/1.73m^2] = 0.413 * (height\ [cm] / S\text{-}cr\ [mg/dL])$$

Abbreviations: GFR (Glomerular filtration rate), cr (creatinine).
$\kappa = 0.7$ for females and 0.9 for males; $\alpha = -0.329$ for females and -0.411 for males; min indicates the minimum of S-cr [*mg/dL*]) / κ or 1; max indicates the maximum of S-cr [*mg/dL*]) / κ or 1; 1 µmol/L = 0.0113 mg/dL and 1 mg/dL = 88.4.

Data analysis and statistical methods

Comparisons of patient characteristics before and after exclusion (in **Table 6-7)** were made using the χ^2 or Fisher's exact test in case of categorical data, or the t- or rank-sum-test in case of continuous parametric and non-parametric variables, respectively. The remaining statistical analyses are described in detail below.

Paper I

To investigate the incidence of early ACR, the percentage distribution of different ACR grades among the early EMBs were calculated. The proportions of early EMBs with ACR ≥ grade 2 were then compared between routine and ACI EMBs. Based on the findings of earlier studies [127-131], similar comparisons were also made between all early EMBs in HTs performed 1988-1999 vs. 2000-2010 ("early" vs. "late" era) and all EMBs obtained week 1-12 vs. 16-52 after HT.

To study predictors of early ACR, the proportions of early EMBs with ACR ≥ grade 2 were compared between the groups of each predictor category defined in **Table 6**.

To study outcome of early ACR, survival was compared in patients with vs. without early ACR ≥ grade 2 and with vs. without early ACR ≥ grade 3A/3B. Two other comparisons were then made to discriminate any potential influence on outcome of ACR of grade 2 from ≥ 3A/3B – namely patients; with one or more early ACR of grade 2 (but no ACR ≥ grade 3A/3B) vs. without early ACR ≥ grade 2, and with one or more early ACR ≥ grade 3A/3B vs. without early ACR ≥ grade 2.

Finally, to gain further insight into the results of the above analyses, patients with vs. without early ACR ≥ grade 2 were compared with regard to cause of death. The proportions of HTs with different types of induction and maintenance therapy at discharge were also compared between 1988-1999 and 2000-2010 ("early" and "late" era).

Except for the survival curves, which were plotted using the Kaplan-Meier method and compared using the log-rank test, all of the above analyses were made using the χ^2 or Fisher's exact test (Fisher's exact test if small sample size).

Paper II

To investigate the incidence of late ACR, calculations were made to obtain the percentage distribution of different ACR grades among late EMBs, the number of late ACRs ≥ grade 2 in relation to the number of late EMBs per year, and the incidence of late ACRs ≥ grade 2 per patient per year. The χ^2 test was specifically used to compare the proportions of late EMBs with ACR ≥ grade 2 between routine and ACI EMBs as well as different eras (1988-1999 vs. 2000-2010). A Kaplan-Meier curve was furthermore constructed to illustrate the proportions of patients free from different ACR grades at different time-points post-HT.

To study predictors of late ACR, relevant variables (recipient age at HT, recipient-donor age difference at HT, recipient gender, sex- and ABO-matching, pre-HT recipient-donor cytomegalovirus [CMV] constellation, first-year ACR ≥

grade 2, ventricular assist device prior to HT, and maintenance immunosuppression at discharge) were analyzed using cox regression. Time to first late ACR ≥ grade 2 was defined as the end-point. Only variables with $p<0.05$ in the univariate model were included in the multivariate model.

To study outcome of late ACR, survival was compared in patients with vs. without late ACR ≥ grade 2 and with vs. without late ACR ≥ grade 3A/3B. As in paper **I**, two other comparisons were also made to discriminate any potential influence on outcome of ACR of grade 2 from ≥ 3A/3B – namely patients; with one or more late ACR of grade 2 (but no ACR ≥ grade 3A/3B) vs. without late ACR ≥ grade 2, and with one or more late ACR ≥ grade 3A/3B vs. without late ACR ≥ grade 2. Cumulative ACRs were then studied by comparing patients with one vs. two or more ACR ≥ grade 3A/3B. For these analyses, the Kaplan-Meier method was used to estimate survival. Group specific comparisons were then made using a time-dependent cox regression model with and without adjustment for age, sex, and first-year ACR ≥ grade 3A/3B.

Paper III

In paper **III**, the incidence of CKD was investigated by constructing a Kaplan-Meier curve, showing the proportions of patients free from different CKD stages before and after HT. Data on renal function and associated parameters (systolic and diastolic blood pressure, body mass index [BMI] and glycated hemoglobin [hba1c] levels) were also plotted, whereupon values at year 1 were compared to transplant assessment (TA), year 5 and year 10. For these analyses, the t- or rank-sum-test were used as appropriate (parametric or non-parametric variables).

To study predictors of CKD, relevant variables pre- and post-HT (recipient age at HT, recipient gender, pre-HT diagnosis, pre-HT systolic and diastolic blood pressure, pre-HT BMI, pre-HT hba1c levels, initiation of TAC / CSA and angiotensin converting enzyme inhibitor / angiotensin receptor blocker by year 1, proteinuria during year 1, and first-year ACR ≥ grade 3A/3B) were separately analyzed using cox regression. Time to >50% GFR reduction was defined as the end-point to study variables associated with a higher rate of GFR decline. In the multivariate models, adjustments were made for previously well identified risk factors for CKD after HT (age, sex, blood pressure and hba1c).

To study outcome of CKD, survival was compared in patients with GFR <60 vs. ≥60 at TA and year 1, as well as in patients with first-year GFR decline of >30% vs. <30%. For these analyses, the Kaplan-Meier method was used to estimate survival. Groups were then compared using cox regression with and without adjustment for age and sex.

Finally, with regard to the accuracy of the CKD-EPI and Schwartz formulae, their estimates were compared with corresponding iohexol clearance measurements

using the paired t-test. In a sub analysis, the Modification of Diet in Renal Disease (MDRD) formula [132] was also validated among the adults. Bland-Altman plots were then created to illustrate estimation differences across different GFR levels.

Paper **IV**

General remarks

As mentioned earlier, the aim of paper **IV** was to study the safety and efficacy of switching stable HT patients to generic immunosuppressants; in this case from CellCept® (Roche, Basel, Switzerland) to Myfenax Teva® (both MMF) and/or from Prograf® (Astellas, Tokyo, Japan) to Tacrolimus Sandoz® (both TAC). For economic reasons, such a switch had been encouraged at SUS-Lund during the fall of 2011. Paper **IV** is an entirely retrospective evaluation of this switch.

Study population

All patients at SUS-Lund who, during the fall of 2011, were treated with CellCept® and/or Prograf®, were considered for inclusion. Out of 61 potential candidates, 55 were included; 38 to Myfenax Teva®, four to Tacrolimus Sandoz®, and 13 to both. Thus, there were a total of 51 and 17 patients switching to Myfenax Teva® and Tacrolimus Sandoz®, respectively. Reasons for exclusion and study population characteristics can be seen in **Fig. 11**.

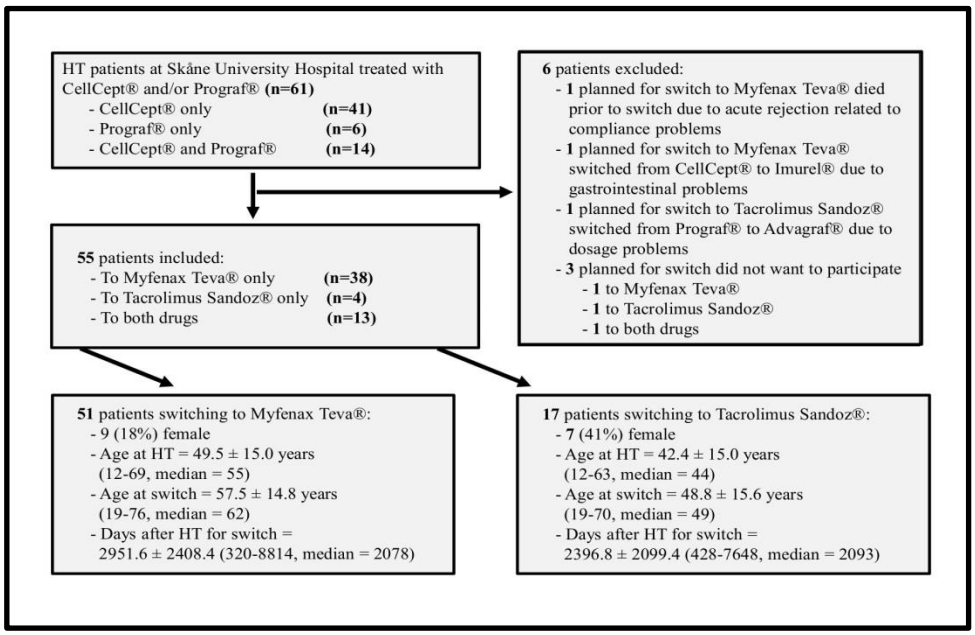


Figure 11.
Reasons for exclusion and study population characteristics of the patients included in paper **IV**.

Acute monitoring

As part of the pre-designed protocol, all patients had undergone an acute monitoring in direct connection to their switch(es). For TAC, therapeutic drug monitoring (TDM) is a standard procedure which has shown to improve both safety and efficacy [133]. TDM could therefore be regarded as a surrogate for these two variables [133]. Area under the curve (AUC) seems to be the key TDM parameter, however, it has been shown that C0 levels correspond well to AUC levels wherefore C0 levels usually are used in clinical practice [134-136]. In patients switching to Tacrolimus Sandoz®, TAC C0 levels had therefore been measured one, two, and three weeks before as well as after the switch.

In contrast to TDM of TAC, TDM of mycophenolic acid (MPA; the measurable metabolite of MMF) has neither shown to improve safety nor efficacy [137-139]. Therefore, the acute monitoring of patients switching to Myfenax Teva® had instead included the gathering of a number of variables reflecting both safety and efficacy. Whereas safety had been monitored by measuring concentrations of hemoglobin, leukocytes and thrombocytes one week before as well as one, two, and three weeks after the switch, efficacy had been monitored with EMB four weeks after the switch. All EMBs had been graded according to the 2004-ISHLT-WF [20] (**Table 1**). In

patients with ACR, additional EMBs had been performed until complete resolution of rejection. Exceptions had however been made if this was considered unnecessary from a clinical viewpoint.

Data analysis and statistical methods

Data from the acute monitoring were retrospectively collected. Concentrations of TAC C0 levels, hemoglobin, leukocytes and thrombocytes were then compared using the paired t- or signed-rank-test and one-way ANOVA or ANOVA on ranks (for parametric and non-parametric data, respectively). The χ^2 test was also used to compare the proportions of EMBs with ACR grade 0R and 1R between patients switching to Myfenax Teva®, and to pre-planned routine EMBs that had been performed in a historical control group selected from the 215 HT patients followed at SUS-Lund 1988-2010 (i.e. the study population paper **I-III** were based on). The inclusion criteria for patients included in this control group can be seen in **Fig. 12**. In total, 44 of the 51 patients switching to Myfenax Teva® had undergone a total of 52 EMBs. As seen in **Fig. 12**, the control group consisted of 24 patients in whom 65 EMBs had been made.

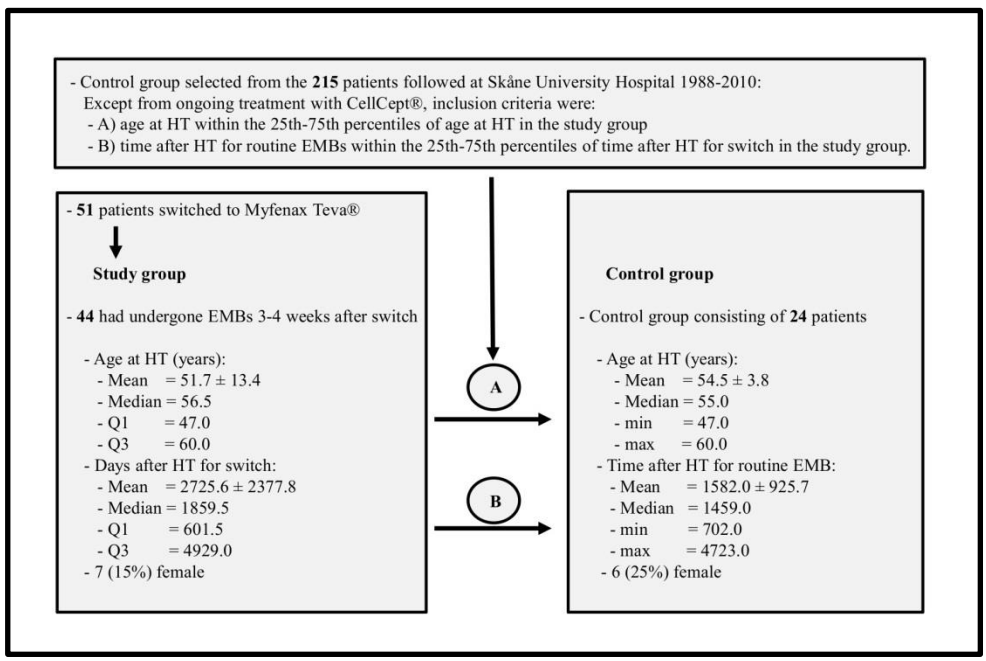


Figure 12.
Selection of control group for comparison of the EMBs performed in the patients switching to Myfenax Teva®.

A long-term follow-up was thereafter conducted. Firstly, data were collected on six month survival, six month history of ACR, and whether or not the patients still were taking Tacrolimus Sandoz® and/or Myfenax Teva® after six months. Secondly, as HT patients at SUS-Lund undergo extensive follow-up once a year, relevant safety data from the annual visits before and after the switches (GFR according to iohexol clearance measurement, systolic and diastolic blood pressure, cholesterol, triglycerides and hba1c for patients switching to Tacrolimus Sandoz®, as well as hemoglobin, leukocytes and thrombocytes for patients switching to Myfenax Teva®) were compared using the paired t- or signed-rank-test (for parametric and non-parametric data, respectively). Finally, TAC C0 levels during the three weeks prior to the switch were also compared to TAC C0 levels during the next annual follow-up. For this analysis, the paired t- or signed-rank-test was also used as appropriate.

Ethics, statistical software and level of significance

Ethical approval was obtained from the ethics board in Lund (Approval nos. 2011/77, 2011/368 and 2010/114). All studies were conducted in accordance with the declarations of Helsinki and Istanbul. With the exception of the time-dependent cox regression analysis in paper **II**, performed in SPSS version 24.0.0.0. (IBM, Armonk, NY), all statistical analyses were made using SigmaStat/SigmaPlot version 11.2.0.5. (Systat Software Inc, San Jose, CA). A significance level of 0.05 was used throughout.

Results

Paper **I**

Incidence of early ACR

The percentage distribution of different ACR grades among the early EMBs is shown in **Fig. 13**. As seen, there were proportionally more ($p<0.05$) ACRs ≥ grade 2 among ACI than routine EMBs. Notably, among all 2990 early EMBs, only two showed grade 3B and only one showed grade 4. Among the 205 HTs where survival was > 25 days and at least one early EMB had been performed, ACRs ≥ grade 1A/1B, 2 and 3A/3B were found at frequencies of 90%, 56% and 38%, respectively.

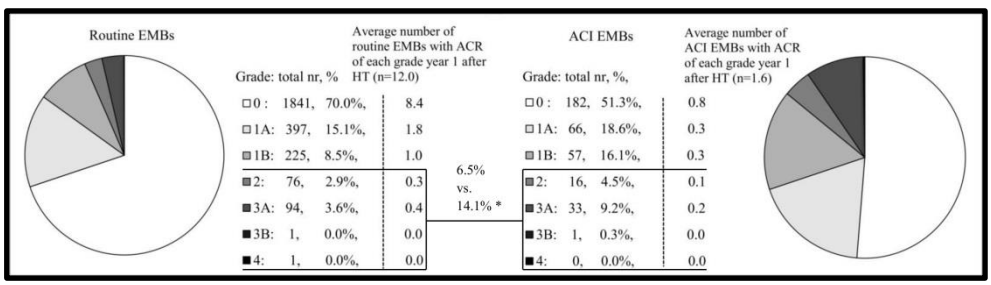


Figure 13.
The percentage distribution of different ACR grades among the early EMBs. * Indicates statistical significance ($p<0.05$).

The percentage distribution of different early ACR grades in relation to year of, and week after, HT, is shown **Fig. 14**. As seen, there were proportionally more ($p<0.05$) ACRs ≥ grade 2 among EMBs (routine and ACI combined) obtained; in HTs 1988-1999 than 2000-2010, and week 16-52 than 1-12.

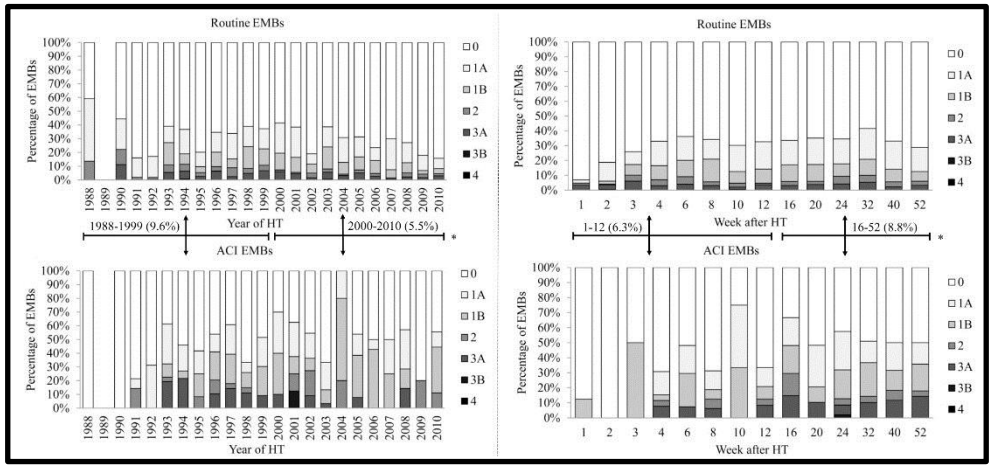


Figure 14.
The percentage distribution of different early ACR grades in relation to year of, and week after, HT. * Indicates statistical significance ($p<0.05$).

Predictors of early ACR

In **Table 9**, the comparison of proportions of early EMBs with ACR ≥ grade 2 between groups of different predictor categories is shown. As seen, there were proportionally more ($p<0.05$) ACRs ≥ grade 2 among EMBs in HTs with pediatric than adult donors and EMBs in sex-mismatched than sex-matched HTs.

Table 9.
Comparison of proportions of early EMBs with ACR ≥ grade 2 between groups of different predictor categories.

Potential predictors	EMBs with ACR ≥ grade 2		
	n	%	p
Age of recipient at HT (years) =			
Pediatric recipients (below 18 years of age)	38	**9.4**	**0.13**
Adult recipients (18 years or older)	184	**7.1**	
Age of donor at HT (years) =			
Pediatric donors (below 18 years of age)	25	**11.3**	**<0.05***
Adult donors (18 years or older)	197	**7.1**	
Difference in age between recipient and donor (years +/-)			
0-11	134	**7.6**	**0.20**
12-22	38	**5.9**	
23-33	39	**9.3**	
34-45	11	**6.6**	
Gender of recipient			
Male	73	**7.5**	**1.00**
Female	149	**7.4**	
Gender of donor			
Male	129	**7.1**	**0.40**
Female	93	**8.0**	
Sex-matching between recipient and donor			
Sex-matched	138	**6.3**	**<0.05***
Sex-mismatched	84	**10.4**	
ABO-matching between recipient and donor			
ABO-identical	188	**7.4**	**0.98**
ABO-compatible	31	**7.4**	
ABO-incompatible	3	**8.3**	
CMV-constellation			
Donor + / Recipient +	101	**6.7**	**0.22**
Donor - / Recipient -	32	**10.0**	
Donor + / Recipient -	25	**7.0**	
Donor - / Recipient +	48	**7.6**	
Recipient diagnosis			
Arrythmogenic right ventricular dysplasia	2	**7.7**	**0.87**
Dilated cardiomyopathy caused by adriamycin	4	**9.3**	
Dilated cardiomyopathy caused by aortic stenosis or insufficiency	15	**10.8**	
Dilated cardiomyopathy	96	**7.1**	
Hypertrophic cardiomyopathy	11	**7.0**	
Right heart failure with ventricular tachycardia or fibrillation	0	**0.0**	
Ischemic heart disease	55	**7.1**	
Chronic vascular rejection	2	**11.8**	
Myocarditis	15	**7.1**	
Restrictive cardiomyopathy	8	**8.6**	
Cardiac tumor	1	**12.5**	
Congenital heart disease	13	**8.8**	
Waiting time (days)			
0-322	187	**7.3**	**0.31**
323-644	29	**9.2**	
645-966	6	**6.7**	
967-1289	0	**0.0**	
Ischemic time (minutes)			
46-126	41	**6.2**	**0.13**
127-207	86	**7.5**	
208-288	91	**8.5**	
289-369	1	**1.9**	
Induction immunosuppression			
Antithymocyte globulin	212	**7.9**	**0.87**
Daclizumab	7	**6.6**	
No induction	2	**6.9**	
Maintenance immonosuppression in patients alive at discharge			
Cyclosporine + Azathioprine + Corticosteroids	103	**7.3**	**0.23**
Cyclosporine + MMF/MPA + Corticosteroids	99	**8.2**	
Tacrolimus + MMF/MPA + Corticosteroids	7	**4.0**	
Other combinations	13	**6.8**	

*Indicates statistical significance (P<0.05). Abbreviations: EMB (Endomyocardial biopsy), ACR (Acute cellular rejection), n (Number of EMBs with ACR≥grade 2 for each group), HT (Heart transplantation), CMV (Cytomegalovirus), MMF/MPA (Mycophenolate mofetil/ acid).

Outcome of early ACR

Survival after HT for all 215 HT patients followed 1988-2010, as well as survival in relation to early ACR, is shown in **Fig. 15**. As seen, overall survival was high (93%, 85% and 74% at year 1, 5 and 10, respectively). Long-term survival (five and 10 years) was lower ($p<0.05$) in patients; with than without early ACR ≥ grade 2, with than without early ACR ≥ grade 3A/3B, and with one or more early ACR ≥ grade 3A/3B than without early ACR ≥ grade 2. However, when comparing patients without early ACR ≥ grade 2 vs. patients with one or more early ACR of grade 2 (but no early ACR ≥ grade 3A/3B), no significant difference was found. Neither were there any significant differences in terms of short-term survival (one year) between any of the groups.

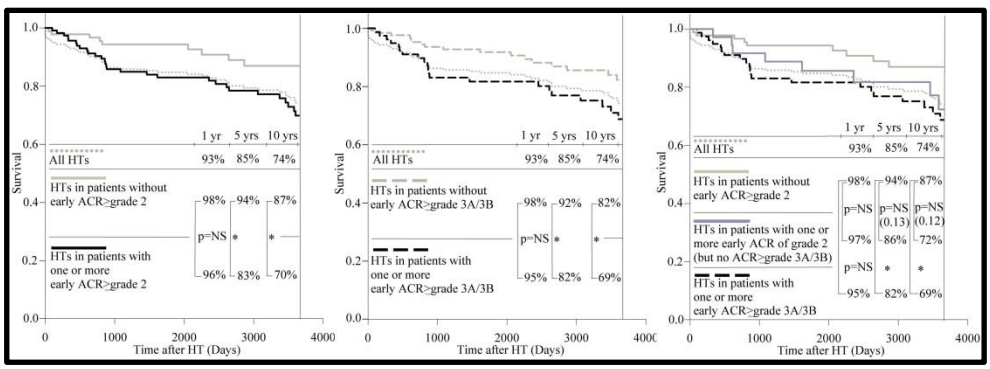


Figure 15.
Survival after HT for all 215 HT patients followed 1988-2010, as well as survival in relation to early ACR. NS = not significant. * Indicates statistical significance ($p<0.05$).

Early ACR and cause of death

In total, 72 of the 215 HT patients (33%) died before the end of follow-up 30th June 2012. The causes of death were as follows; primary graft failure (PGF; 6%), multiple organ failure (MOF; 6%), rejection (17%), malignancy (26%), infection (8%), CKD (6%) and "other" (32%). After exclusion of 11 patients in whom no early EMBs had been performed (those with PGF and MOF), the causes of death did not differ (p=NS) between patients with vs. without early ACR ≥ grade 2.

Immunosuppression 1988-1999 vs. 2000-2010

Induction and maintenance therapy at discharge 1988-1999 vs. 2000-2010 can be seen in **Fig. 9**. Although ATG and CSA in total were given more often than daclizumab and TAC, respectively, there was from 1988-1999 to 2000-2010 an increase ($p<0.05$) in the use of daclizumab and TAC + MMF/MPA + CSs, but a decrease ($p<0.05$) in the use of CSA + AZA + CSs.

Paper **II**

Incidence of late ACR

Among all 815 late EMBs, the following distribution of ACR grades was found – grade; 0: 60.1% (n=490), 1A: 15.2% (n=124), 1B: 13.3% (n=108), 2: 4.3% (n=35), 3A: 6.5% (n=53), 3B: 0.5% (n=4), and 4: 0.1% (n=1). The percentage distribution according to indication (routine or ACI EMBs) is shown in **Fig. 16**. As seen, there were proportionally more ($p<0.05$) ACRs ≥ grade 2 among ACI than routine EMBs. No difference was however found (p=NS) when comparing the proportions of EMBs (routine and ACI combined) with ACR ≥ grade 2 between EMBs obtained 1988-1999 (14.0%) vs. 2000-2010 (10.1%).

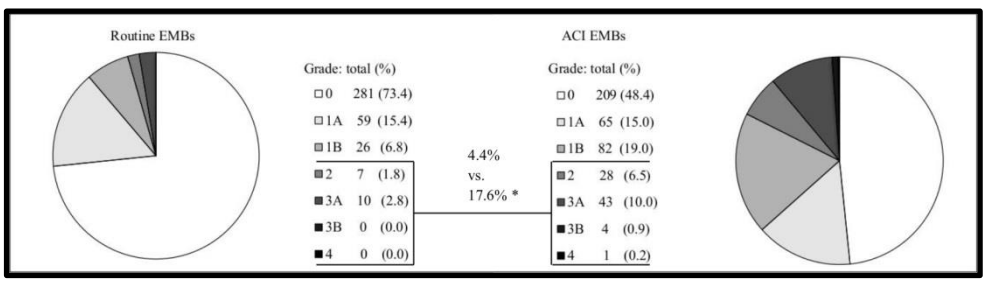
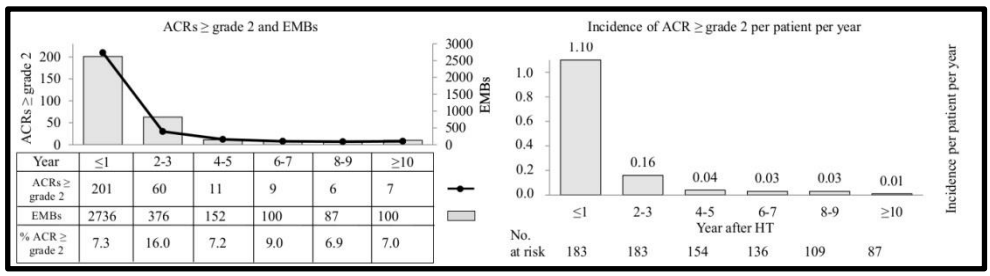


Figure 16.
The percentage distribution of different ACR grades among the late routine and ACI EMBs. * Indicates statistical significance ($p<0.05$).

Fig. 17 illustrates the number of ACRs ≥ grade 2 in relation to the number of EMBs, and the incidence of ACR ≥ grade 2 per patient per year, post-HT. As seen, although the proportion of EMBs showing ACR ≥ grade 2 remained relatively stable (except for possibly year 2-3), there was a clear trend with time post-HT towards fewer ACRs ≥ grade 2 and a lower incidence of ACR ≥ grade 2 per patient per year.

The sharpest decrease in both the number and incidence of ACRs ≥ grade 2 was from year 1 to year 2-3. The changes during the years thereafter were comparatively small. Of notice, however, the only late EMB showing ACR grade 4 was also the EMB obtained the longest time post-HT in the entire study (18 years). This patient presented with acute heart failure requiring inotropic support and died only shortly following initiation of rejection therapy.



Year	≤1	2-3	4-5	6-7	8-9	≥10
ACRs ≥ grade 2	201	60	11	9	6	7
EMBs	2736	376	152	100	87	100
% ACR ≥ grade 2	7.3	16.0	7.2	9.0	6.9	7.0

Figure 17.
The number of ACRs ≥ grade 2 in relation to the number of EMBs, and the incidence of ACR ≥ grade 2 per patient per year, post-HT.

Fig. 18 illustrates the proportions of patients free from different ACR grades at various time-points post-HT. As seen, the sharpest decreases were during year 1 post-HT, regardless of grade and the cumulative number of ACRs. Beyond the first year, there were continued decreases, but only small in comparison.

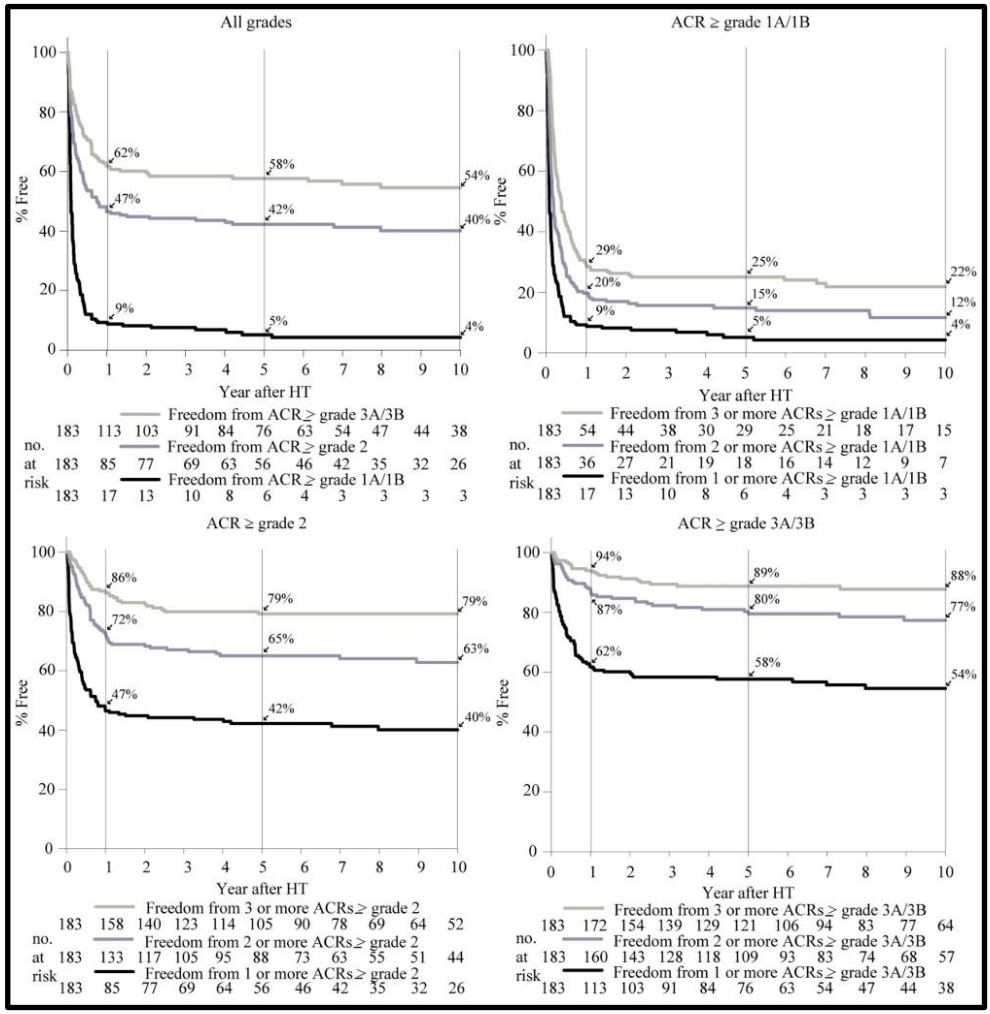


Figure 18.
The proportions of patients free from different ACR grades at various time-points post-HT.

Predictors of late ACR

Potential predictors of late ACR ≥ grade 2 are shown in **Table 10**. As seen in the univariate model, variables associated ($p<0.05$) with an increased risk of late ACR ≥ grade 2 were younger recipient age, sex-mismatching and history of first-year ACR ≥ grade 2. However, following multivariate analysis, only sex-mismatching and history of first-year ACR ≥ grade 2 remained significant.

Table 10.
Potential predictors of late ACR ≥ grade 2.

	Univariate cox-regression			Multivariate cox-regression		
	HR	95% CI	p	HR	95% CI	p
Recipient-donor characteristics						
Recipient age (years)	0.98	0.96-1.00	<0.05*	0.98	0.97-1.00	0.05
Recipient-donor age difference (years)	1.01	0.98-1.03	0.72	-	-	-
Recipient gender (female)	1.22	0.65-2.27	0.54	-	-	-
Sex-mismatch	2.81	1.53-5.16	<0.05*	2.29	1.23-4.27	<0.05*
Not ABO-identical	1.00	0.44-2.25	1.00	-	-	-
CMV + donor and CMV - recipient	0.73	0.26-2.06	0.55	-	-	-
History of first-year ACR ≥ grade 2	3.55	1.69-7.41	<0.05*	3.29	1.57-6.90	<0.05*
VAD prior to HT	0.66	0.33-1.35	0.26	-	-	-
Immunosuppression at discharge						
Cyclosporine + Azathioprine + Corticosteroids vs. Cyclosporine + MMF/MPA + Corticosteroids	0.96	0.50-1.83	0.90	-	-	-
Tacrolimus + MMF/MPA + Corticosteroids vs. Cyclosporine + Azathioprine + Corticosteroids	1.84	0.54-6.29	0.33	-	-	-
Tacrolimus + MMF/MPA + Corticosteroids vs. Cyclosporine + MMF/MPA + Corticosteroids	2.21	0.63-7.80	0.22	-	-	-

*Indicates statistical significance (P<0.05). Abbreviations: ACR (Acute cellular rejection), HR (Hazard ratio), CI (Confidence interval), CMV (Cytomegalovirus), VAD (ventricular assist device), MMF/MPA (Mycophenolate mofetil/ acid).

Outcome of late ACR

Survival in relation to late ACR is shown in **Fig. 19**. As seen, the risk of death was higher ($p<0.05$) in patients; with than without late ACR ≥ grade 2, with than without late ACR ≥ grade 3A/3B, and with one or more late ACR ≥ grade 3A/3B than without late ACR ≥ grade 2. However, when comparing patients without late ACR ≥ grade 2 vs. patients with one or more late ACR of grade 2 (but no late ACR ≥ grade 3A/3B), no significant difference was found. Neither was there any significant difference between those with one vs. two or more late ACR ≥ grade 3A/3B.

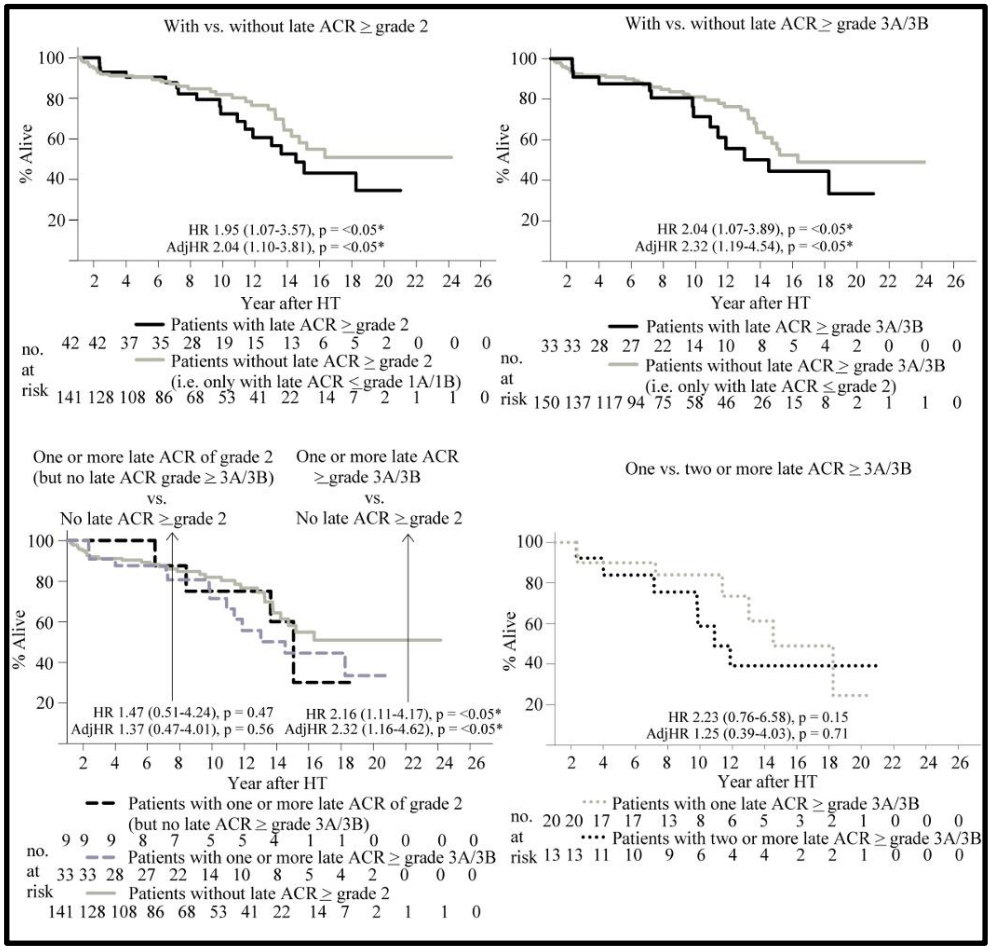


Figure 19.
Survival in relation to late ACR. Values within parentheses represent 95% confidence intervals. * Indicates statistical significance (p<0.05).

Paper **III**

Incidence of CKD

During a mean follow-up of 8.3±5.7 (0.0-24.2) years, 46 (34%) patients died. Acute on chronic renal failure was one of the most important contributing factors in two of those patients (year 2 and 14 post-HT). Thirty-nine (29%) patients reached CKD ≥ stage 4 (GFR < 30 ml/min/1.73m^2; median=3, min=0, max=12 years) and 14

(10%) reached CKD stage 5 (GFR < 15 ml/min/1.73m^2; median=9, min=0, max=17 years). Ten (7%) required dialysis and three (2%) a renal transplant. The renal transplants were performed 10, 13 and 20 years post-HT.

In **Fig. 20**, the proportions of patients free from different CKD stages before and after HT are shown. As seen, a large proportion of the patients had a significant renal function impairment prior to HT. In the first year, a seemingly larger proportion of the patients progressed above CKD stage 2 and 3 than 4 and 5. In the following years, the proportions of patients with any CKD stage steadily increased.

Fig. 20 moreover illustrates GFR during TA and over the 10 first years post-HT. As seen, one year post-HT, GFR was lower ($p<0.05$) than during TA. It thereafter declined at a slower, yet steady rate, to a significantly lower ($p<0.05$) level at year 10. Of interest, mean yearly GFR change during year 1 was -11.9±25.8 ml/min/1.73 m^2 as compared to -2.2±14.6 ml/min/1.73m^2 during the follow-up as a whole.

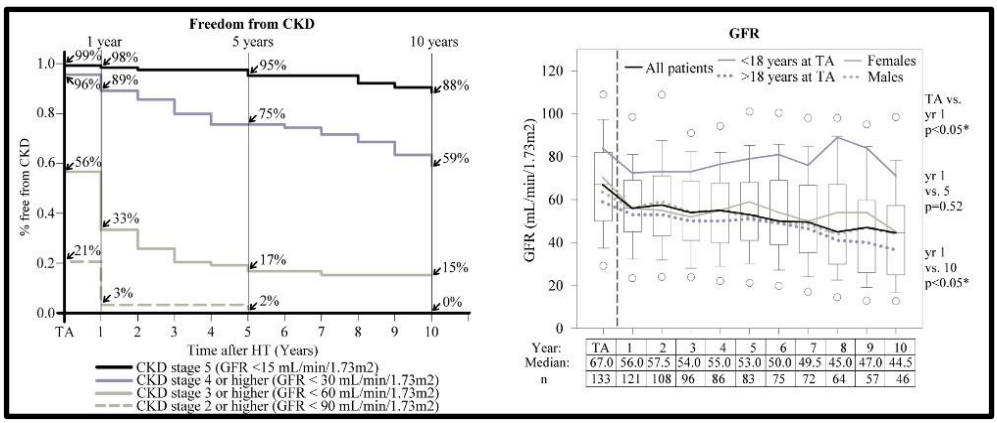


Figure 20.
The proportions of patients free from different CKD stages before and after HT, as well as GFR during TA and over the 10 first years post-HT. * Indicates statistical significance ($p<0.05$).

In **Fig. 21**, measured and estimated GFR, as well as associated parameters during TA and over the 10 first years post-HT, are shown. As seen, S-creatinine and S-urea increased over time and reached slightly higher ($p<0.05$) levels at year 10 compared to year 1. A clear increase ($p<0.05$) was also seen in systolic and diastolic blood pressure during the first year. However, neither systolic nor diastolic blood pressure changed much in the years thereafter. Similarly, BMI and hba1c remained relatively stable following HT, although an increase in BMI and a decrease in hba1c was found when values at TA and year 1 were compared ($p<0.05$).

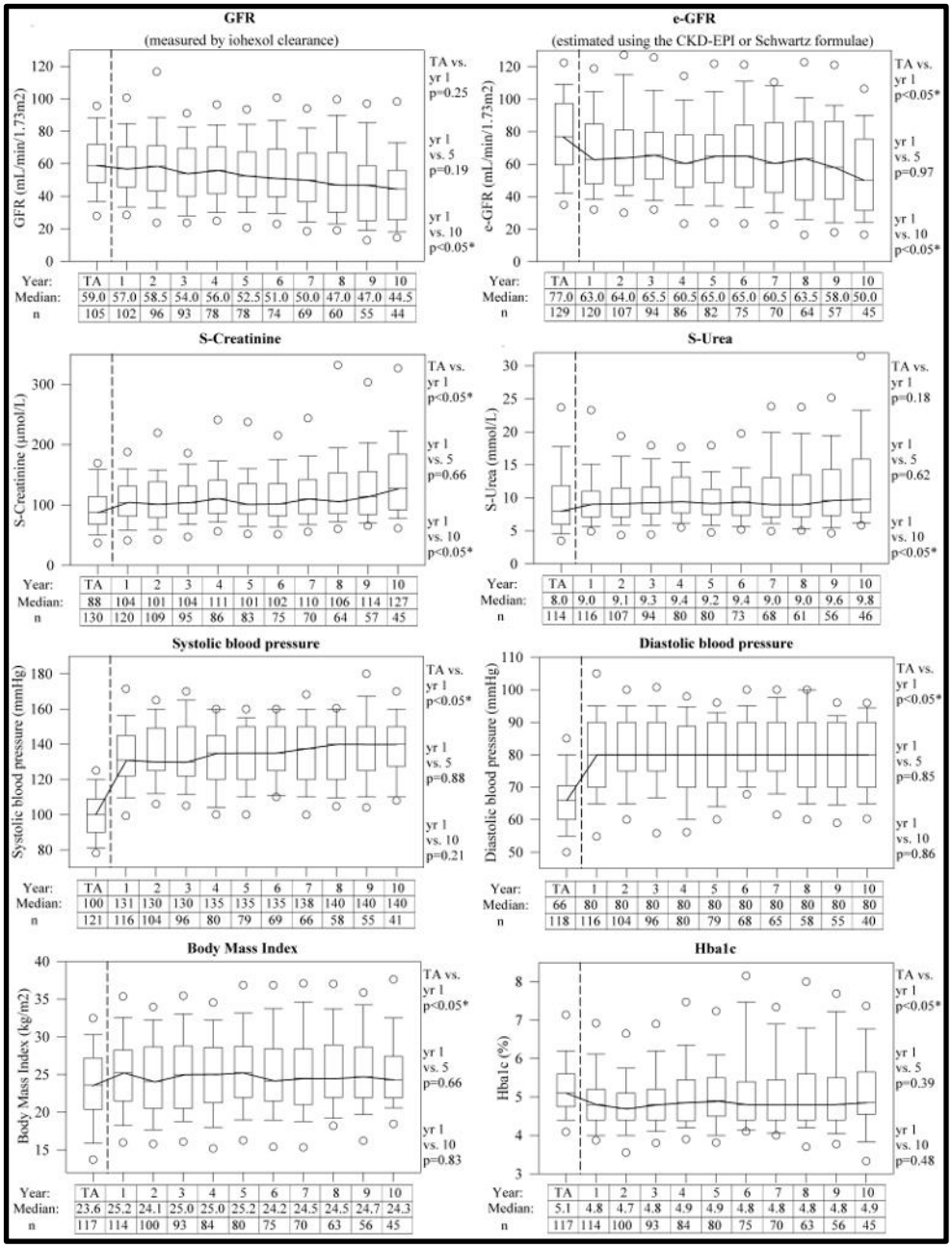


Figure 21.
Measured and estimated GFR, as well as associated parameters during TA and over the 10 first years post-HT. * Indicates statistical significance (p<0.05).

Predictors of CKD

Worsening in renal function in relation to potential pre- and post-HT predictors is shown in **Table 11** and **12**. As seen, the only variable related ($p<0.05$) to a higher rate of GFR decline was proteinuria during year 1.

Table 11.
Potential pre-HT predictors of CKD.

Potential pre-HT predictors	Univariate cox-regression			Multivariate cox-regression		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Recipient age (years)	1.01	(0.99-1.03)	0.24	1.00	(0.97-1.04)	0.96
Recipient gender (female)	1.09	(0.57-2.09)	0.80	1.86	(0.68-5.05)	0.23
Pre-HT diagnosis (ICM)	0.97	(0.48-1.98)	0.94	0.71	(0.24-2.08)	0.53
Pre-HT SBP (mmHg)	1.02	(0.99-1.04)	0.08	1.02	(0.99-1.05)	0.17
Pre-HT DBP (mmHg)	1.03	(0.99-1.07)	0.09	1.00	(0.94-1.05)	0.91
Pre-HT BMI (kg/m^2)	1.03	(0.96-1.10)	0.44	0.97	(0.86-1.10)	0.61
Pre-HT hba1c (%)	0.79	(0.47-1.31)	0.36	0.80	(0.47-1.37)	0.42

*Indicates statistical significance (P<0.05). Abbreviations: HT (Heart transplantation), HR (Hazard ratio), CI (Confidence interval), ICM (Ischemic cardiomyopathy), SBP/DBP (Systolic/diastolic blood pressure), BMI (Body mass index), hba1c (Glycated hemoglobin).

Table 12.
Potential post-HT predictors of CKD.

Potential post-HT predictors	Univariate cox-regression			Multivariate cox-regression		
	HR	**95% CI**	**p**	**HR**	**95% CI**	**p**
Initiation of TAC as compared to CSA by year 1[a]	1.07	(0.41-2.78)	0.89	0.42	(0.05-3.58)	0.43
Initiation of ACEI or ARB by year 1	1.24	(0.68-2.28)	0.49	0.99	(0.43-2.30)	0.98
Proteinuria at year 1	2.46	(1.04-5.82)	<0.05*	5.15	(1.23-21.55)	<0.05*
First-year ACR ≥ grade 3A/3B[b]	1.30	(0.71-2.36)	0.40	1.58	(0.68-3.65)	0.29

*Indicates statistical significance (P<0.05). Abbreviations: HT (Heart transplantation), HR (Hazard ratio), CI (Confidence interval), TAC (Tacrolimus), CSA (Cyclosporine), ACEI (Angiotensin converting enzyme inhibitor), ARB (angiotensin receptor blocker), ACR (acute cellular rejection).
[a] after exclusion of patients also treated with everolimus, and those receiving both CSA and TAC
[b] after exclusion of patients who did not undergo endomyocardial biopsy during year 1.

Outcome of CKD

Survival during the 10 first years after HT in relation to GFR at TA, GFR at year 1, and in relation to first-year change in GFR, is shown in **Fig. 22**. As seen, the risk of death was lower ($p<0.05$) in patients with; GFR ≥60 as compared to <60 ml/min/1.73m^2 at TA, and first-year GFR decline of <30% as compared to >30%.

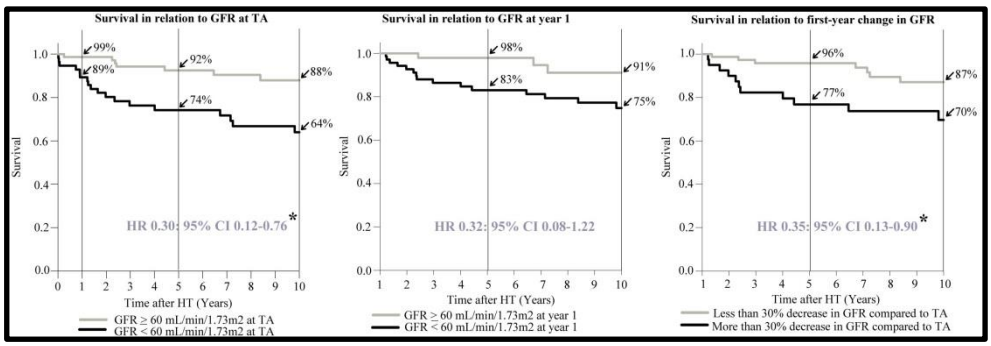


Figure 22.
Survival during the 10 first years after HT in relation to GFR at TA, GFR at year 1, and in relation to first-year change in GFR. * Indicates statistical significance ($p<0.05$).

Accuracy of CKD-EPI and Schwartz formulae

Compared with measured GFR, GFR (mean ± SD in ml/min/1.73m^2) was higher ($p<0.05$) using the CKD-EPI (62.1±24.8 vs. 50.0±20.1, n=818) or Schwartz (94.5±22.6 vs. 79.0±24.4, n=88) formula. On average, the CKD-EPI formula overestimated measured GFR by 12.2±13.2 ml/min/1.73m^2 (27.9±28.7%) and misclassified 44% of all cases to a more advanced CKD stage (vs. 2% to less advanced). Similarly, the Schwartz formula overestimated measured GFR by 15.5±19.6 ml/min/1.73m^2 (25.9±32.7%) and misclassified 44% of all cases to a more advanced CKD stage (vs. 3% to less advanced). Notably, only 59.2% and 59.1% of the estimates of each equations, respectively, were within ±30% of measured GFR. In the sub analysis of the MDRD equation, estimated GFR was also higher ($p<0.05$) than measured GFR (61.9±25.4 vs. 50.0±20.1, n=818). Mean bias was +12.0±15.4 ml/min/1.73m^2 (28.2±31.6%), there was a 43% "overclassification" vs. a 2% "underclassification", and only 59.2% of the estimates were within ±30% of true GFR. The estimation errors across different GFR levels are illustrated in Bland-Altman plots in **Fig. 23**. As seen, the positive estimation errors seemed to be relatively persistent at all GFR levels, perhaps more pronounced at higher GFR levels for CKD-EPI and MDRD.

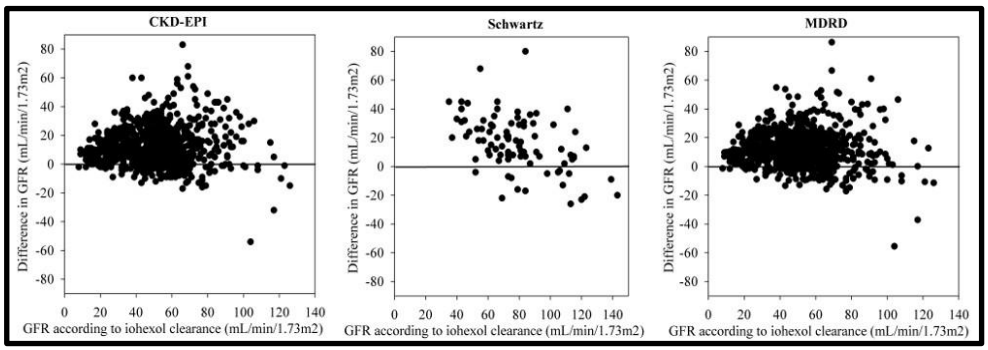


Figure 23.
Bland-Altman plots showing the estimation errors across different GFR levels.

Paper **IV**

Acute monitoring

Prograf® to Tacrolimus Sandoz®

There was no difference (p=0.356) between mean TAC C0 levels taken during the three weeks before (8.4±1.9 µg/l) and after (8.7±2.9 µg/l) the switch to Tacrolimus Sandoz® (paired t-test) (**Fig. 24**). Neither were there any differences (p=0.974) in median TAC C0 levels between any of the individual weeks (ANOVA on ranks).

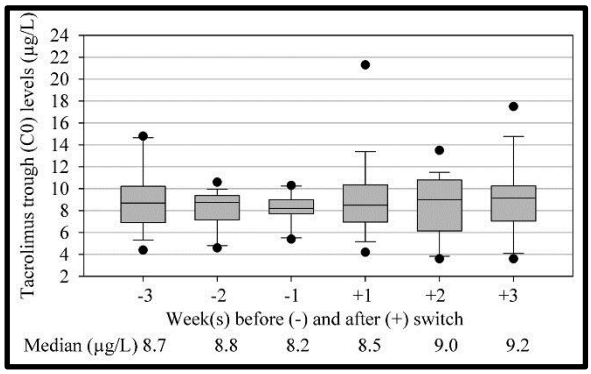


Figure 24.
TAC C0 levels during the acute monitoring of the switch to Tacrolimus Sandoz®. The box-plot graph shows the median, 10th, 25th, 75th and 90th percentiles of TAC C0 levels.

CellCept® to Myfenax Teva®

Hemoglobin, leukocytes and thrombocytes were unchanged (p=NS) when comparing blood samples one week before and three weeks after the switch to Myfenax Teva® (**Fig. 25**).

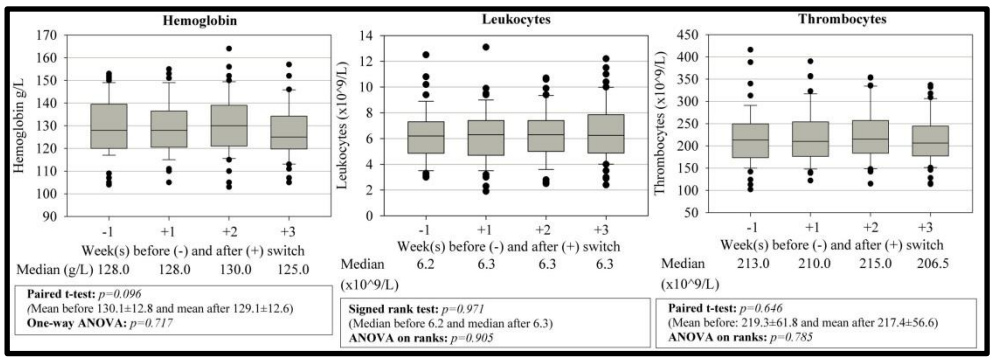


Figure 25.
Hemoglobin, leukocytes and thrombocytes during the acute monitoring of the switch to Myfenax Teva®.

The frequency and severity of ACR seen on the EMBs taken after the switch to Myfenax Teva® were low and did not differ (p=NS) from the control group (**Fig. 26**).

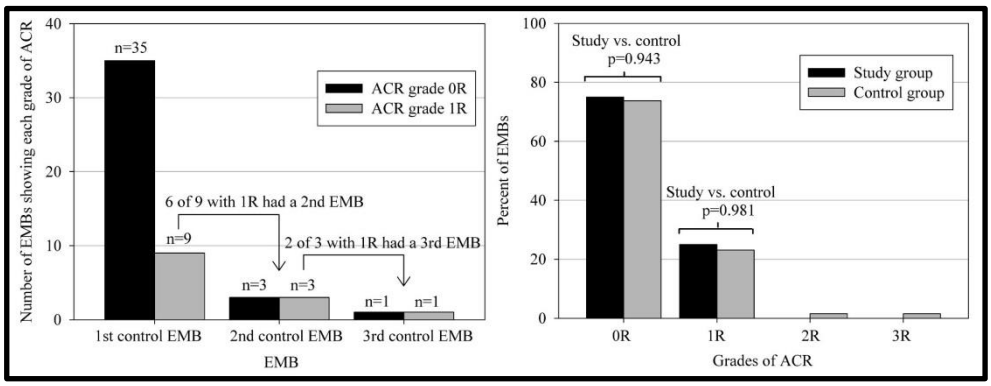


Figure 26.
ACRs on EMBs taken during the acute monitoring of the switch to Myfenax Teva®.

Survival, ACR and generic drug adherence after six months

Prograf® to Tacrolimus Sandoz®

Among those switching to Tacrolimus Sandoz®, survival was 100% (17/17) six months after the switch. In these 17 patients during these six months, eight EMBs were performed in six of the patients due to a concurrent switch to Myfenax Teva®. Of these eight EMBs, four showed grade 0R and four showed grade 1R. Apart from this, no patients required additional EMBs due to suspicion of ACR within six months after the conversion. Moreover, six months after the switch, 94% (16/17) remained on Tacrolimus Sandoz®. One of the 17 patients was switched to Advagraf® 151 days after the switch due to unstable TAC C0 levels.

CellCept® to Myfenax Teva®

Among those switching to Myfenax Teva®, survival was 96% (49/51) six months after the switch. Both patients who died were still on Myfenax Teva® at the time of death. One of the deaths (day 136) was due to pulmonary fibrosis. The cause of death in the second patient (day 166) is however uncertain. This patient presented with dyspnea, elevated NT-proBNP (N-terminal of the prohormone brain natriuretic peptide) and troponin T levels, together with signs of left ventricular dysfunction on echocardiography 110 days after the switch. As there was initial suspicion of rejection, the patient was given rejection treatment with high-dose intravenous CSs and ATG upon hospital admission. However, neither of the two EMBs obtained shortly thereafter confirmed this diagnosis. Moreover, antibody screening only detected low levels of donor-specific antibodies against HLA-DQ5. The post-mortem examination showed no evidence of rejection or infarction. A non-significant stenosis of the proximal part of the right coronary artery was however found. This was consistent with a coronary angiography and a myocardial scintigraphy performed 159 days before and five days after admission, respectively.

Apart from the EMBs obtained in connection with the acute monitoring, only the patient described above required additional EMBs due to suspicion of ACR within six months after the conversion. Moreover, six months after the switch, 96% (47/49) remained on Myfenax Teva®. Two of the 49 patients were switched back to CellCept® 122 and 166 days after the switch due to gastrointestinal problems.

Safety parameters and TAC C0 levels at the next annual follow-up

Safety parameters before and during the next annual follow-up after each switch, are shown in **Table 13**. As seen, all safety parameters were stable except from leukocytes, which decreased from 6.5 to 5.8 x 10^9/L ($p<0.05$) after the switch to Myfenax Teva®.

Table 13.
Safety parameters before and during the next annual follow-up after each switch.

Parameter	n	Time before switch Mean ± SD (range)	Time after switch Mean ± SD (range)	Before switch Mean ± SD	After switch Mean ± SD	p
Prograf® to Tacrolimus Sandoz®						
GFR according to iohexol clearance measurement (mL/min/1.73m^2)	13/17	Scheduled annual follow-up 183.4 ± 104.1 (19-337) days before switch	Scheduled annual follow-up 179.7 ± 104.8 (25-359) days after switch	66.2 ± 21.3	66.8 ± 25.1	0.880
SBP (mmHg)	10/17	Scheduled annual follow-up 192.4 ± 105.7 (50-337) days before switch	Scheduled annual follow-up 169.9 ± 104.1 (25-316) days after switch	125.2 ± 16.5	125.0 ± 14.3	0.961
DBP (mmHg)	10/17	Scheduled annual follow-up 192.4 ± 105.7 (50-337) days before switch	Scheduled annual follow-up 169.9 ± 104.1 (25-316) days after switch	79.2 ± 7.4	78.9 ± 7.7	0.889
Total cholesterol (mmol/L)	13/17	Scheduled annual follow-up 184.9 ± 108.2 (19-337) days before switch	Scheduled annual follow-up 178.0 ± 108.9 (25-359) days after switch	4.9 ± 1.4	4.4 ± 1.2	0.118
Triglycerides (mmol/L)	13/17	Scheduled annual follow-up 184.9 ± 108.2 (19-337) days before switch	Scheduled annual follow-up 178.0 ± 108.9 (25-359) days after switch	1.3 (Median)	1.3 (Median)	0.123
Hba1c (mmol/mol)	14/17	Scheduled annual follow-up 183.4 ± 104.1 (19-337) days before switch	Scheduled annual follow-up 179.7 ± 104.8 (25-359) days after switch	39.0 ± 6.3	40.1 ± 8.4	0.199
CellCept® to Myfenax Teva®						
Hemoglobin (g/L)	40/51	One week before switch	Scheduled annual follow-up 162.6 ± 98.3 (24-404) days after switch	130.1 ± 12.8	127.0 ± 14.8	0.060
Leukocytes (x 10^9/L)	40/51	One week before switch	Scheduled annual follow-up 162.6 ± 98.3 (24-404) days after switch	6.5 ± 2.1	5.8 ± 2.0	**0.004***
Thrombocytes (x 10^9/L)	41/51	One week before switch	Scheduled annual follow-up 160.4 ± 98.1 (24-404) days after switch	218.8 ± 62.7	212.1 ± 63.8	0.287

*Indicates statistical significance ($p<0.05$). Abbreviations: SD (Standard deviation), GFR (glomerular filtration rate), SBP (Systolic blood pressure), DBP (Diastolic blood pressure), Hba1c (glycated hemoglobin).

Finally, no difference (p=0.626) was found in median TAC C0 levels between blood samples taken during the three weeks before switching to Tacrolimus Sandoz® (8.35 µg/l) vs. the next annual follow-up (8.25 µg/l) 179.7±104.8 days after the switch (n=14).

Discussion

Paper **I**

The risk of death is highest the first year post-HT. Since 10% of these deaths are caused by ACR [10], increased understanding of early ACR is vital.

Two of the major findings in Paper **I** were that early ACR of at least grade 3A/3B appeared to influence long-term survival (five and 10 years) and that overall 10-year survival was as high as 74%, as compared to 53% in the ISHLT registry [10]. As opposed to long-term, short-term survival (one year) was not affected by early ACR. Although not specifically analyzed, it is possible that this partly could be explained by subsequent development of CAV. As previously described, CAV is a major cause of late death [16], and has also been linked to ACR earlier [139-141]. The idea that early ACR contributes to late mortality through CAV is not supported by the finding that the causes of death were unrelated to first-year ACR ≥ grade 2. However, few patients died and the data on the causes of death were mostly based on clinical diagnoses rather than autopsies. Patients who died following clinical signs of myocardial infarction had been categorized as "other", and it is possible that some of these patients died of previously undiagnosed CAV.

The frequency and severity of early ACR was low. When analyzed in relation to time of, and after, HT, there were proportionally more early ACRs ≥ grade 2 among EMBs obtained; in HTs 1988-1999 than 2000-2010, and week 16-52 than 1-12. A higher frequency of early ACR in the "early" than the "late" era has also been observed previously [127-128]. This may, at least partly, be explained by time-related developments in immunosuppression, also seen at SUS-Lund. However, contrary to the findings, a few earlier studies have found a higher ACR frequency month 0-3 than 4-12 post-HT [129-131]. The reason for this discrepancy remains unclear but may at SUS-Lund be associated with the time-related reduction in the number of routine EMBs performed.

Previous studies have linked ACR with HLA-mismatching [129,142], young recipients and donors [129,142-143], female recipients and donors [129,142-143], sex-mismatching [144], CMV infection [145-146], and long ischemic time [129,147]. Other studies have analyzed ACR in relation to different combinations of induction [34-38,41-46,148-153] and maintenance [61,119,154] immunosuppressive therapies. In consistency with some of the studies mentioned,

paper **I** indicated that early ACR ≥ grade 2 was associated with young donors and sex-mismatching. The reason for these associations remains unclear. However, as for sex-mismatching, immune responses driven by sex-chromosome linked minor histocompatibility antigens have previously been suspected [144].

Paper **II**

As described in the introduction, continuation of routine EMBs at later stages than around 12 months post-HT remains debated. A few earlier studies have not only indicated low yields of ACR [129-131,155-159], but also uncertain survival benefits [14,160-161], with such strategy. These observations and accumulated clinical experience, together with potential risks, impact on life quality, and costs associated with the procedure, have made many HT centers (including SUS-Lund) abandon long-term routine EMBs except for in high-risk patients. Still, however, there is no clear consensus on the appropriate time of when to stop performing EMBs on a routine basis. Moreover, the definition of "high-risk" patient in this context remains unclear. In paper **II**, these issues were approached.

Notably, there was a low proportion of late EMBs with ACR ≥ grade 2. With time post-HT, there was a clear trend towards fewer ACRs ≥ grade 2, a lower incidence of ACR ≥ grade 2 per patient per year, and a deceleration in the decrease in the proportion of patients free from ACR, regardless of grade and cumulative numbers of ACR episodes. Although direct comparisons with earlier studies are difficult to make, mainly due to large variations in the definition of ACR, the findings correspond well with previous reports [129-131,155-159].

Despite the reassuring results, a few isolated episodes of serious ACR were observed even many years post-HT. It is also interesting that the proportion of routine EMBs with ACR ≥ grade 2 only was slightly lower among late than early EMBs (4.4% vs. 6.5%). This might indicate that the observed decrease in the number and incidence of ACR ≥ grade 2, at least to a small extent, might have been influenced by the time-related decrease in the number of EMBs performed. In turn, this would suggest that late more often than early ACR remained undetected. The survival analysis furthermore indicated that late, and not only early, ACR of at least grade 3A/3B influenced outcome. This may be the first data supporting such an association. Three earlier studies [14,160-161] have compared a "standard" vs. "restrictive" EMB protocol late post-HT without finding any survival benefits with the former strategy. However, none of them studied outcome in relation to actual episodes of late ACR. Two reports [162-163] also found that untreated late ACR not affected survival and often resolved. However these specifically focused on ACRs of grade 2 and ACRs beyond year 2, respectively.

Looking at predictors of late ACR, the findings indicated that sex-mismatching and history of first-year ACR ≥ grade 2 were associated with an increased risk of late ACR ≥ grade 2. These findings slightly contrast the findings on predictors in paper **I**. Of most earlier studies on predictors of ACR, only few have focused on late ACR in specific. Of those that have, a handful have found a correlation between early and late ACR [128,155-158,164-166], but only one has investigated and subsequently found a correlation between late ACR and sex-mismatching [167]. That early and late ACR may be associated appears logical. As for sex-mismatching, the hypothesis described previously may also apply here.

Paper **III**

With the improvement in survival post-HT over the last few decades, detailed in the introduction, several long-term complications have become evident. CKD is of particular importance. Not only may it depend on traditional risk factors (e.g. hypertension and diabetes), but also factors specific to the pre- (e.g. renal hypoperfusion due to advanced heart failure with hemodynamic compromise), peri- (e.g. acute renal injury due to complications leading to reduction in renal blood flow) and post- (e.g. CNI use) operative period. Its diagnosis is of great importance. Today, most centers rely on creatinine-based estimations of GFR. Although KDIGO in their latest guidelines [124] recommends the CKD-EPI [125] and Schwartz [126] formulae in adults and children, respectively, the validity of these (and other) estimating equations in HT patients has not been fully studied [13]. This issue, together with the lack of consensus and significant variation in the definition of CKD, makes earlier studies difficult to interpret and compare. In paper **III**, ~90% of the GFR data were based on iohexol clearance measurements.

Quite a large proportion of the patients developed severe stages of CKD. For instance, five and 10 years post-HT, the cumulative incidence of CKD ≥ stage 4 was 25% and 41%, respectively. This is slightly higher than in two of the largest studies on CKD after HT, where the corresponding rates were 11% and 13% after five years as well as 18% and 22% after 10 years [168-169]. The findings are somewhat surprising considering the excellent 10-year survival at SUS-Lund (74% vs. 53% in the ISHLT registry). However, contrary to paper **III** where ~90% of the GFR data were based on iohexol clearance measurements, the two studies mentioned above reported data on GFR estimated with the MDRD formula [132]. Although validated among non-transplant patients, a recent systematic review found the MDRD formula to overestimate GFR in HT patients by as much as 8.8% [170]. A tendency to overestimate GFR was also found in paper **III**. It is therefore possible that earlier studies have underestimated the true incidence of CKD after HT.

Looking at the mean yearly GFR change during the follow-up as a whole, the average loss was more than twice as high as compared to what has been reported from large longitudinal studies in healthy subjects [171-172]. In accordance with earlier studies [169,173-181], the steepest decline was observed during the first year. This is likely, in large part, explained by the higher CNI levels patients received during this time period. Finding ways of minimizing CNI use has been the focus of several earlier studies, where induction therapy [26-27] and mTOR inhibitors [66-67,76,103,106] have shown to be useful. In paper **III**, induction therapy and mTOR inhibitors could not be studied due to the low number of patients. The use of renoprotective agents was however evaluated, but none of the different treatment modalities were associated with a higher or lower rate of GFR decline.

In earlier studies, older age [168-169,173-174], female sex [168-169,173-174], hypertension [168] and diabetes [168-169,174] have been associated with an increased risk of renal dysfunction following HT. In paper **III**, the only parameter associated with a higher rate of GFR decline was proteinuria during year 1. Proteinuria has earlier been identified as a marker of GFR decline in the general population [182] and kidney transplant patients [183], but little has been known on its usefulness as a predictor in HT patients.

Other important findings include the association between early renal dysfunction and lower survival rates, confirming data from a few earlier studies [168-169,173-174], and that the CKD-EPI and Schwartz formulae appeared unreliable in estimating GFR. Compared to the few earlier studies that have evaluated the two equations in HT patients [170,184], the findings indicated slightly higher positive bias. Nonetheless, it still appears as if all of the three estimating equations studied tend to overestimate GFR in HT patients. The reason for this remains unclear. Muscle atrophy secondary to long-term CS use, with lower levels of circulating creatinine resulting in higher estimates of GFR, may however contribute.

Notable limitations of paper **I-III**

Notable limitations of paper **I-III** include the retrospective design and the relatively few patients included, of which both entail a cautious approach in the interpretation of the findings - especially on predictors and outcome. Moreover, lack of data, which is a direct consequence of the retrospective design, made it impossible to include a number of variables that would have been of interest (e.g. other predictors, AMR and CAV). Other limitations are detailed in each paper, found in the appendix.

Paper **IV**

As many patents have expired, several different generic immunosuppressants have become available. In general, generic drugs are required to be identical to branded drugs with respect to their active ingredients, strengths, doses, administration routes, and indications. They may however differ in the inactive ingredients, shape, color, release mechanism, and packaging [185-187]. Moreover, for approval, manufacturers must show through bioequivalence studies that their generic drug has similar pharmacokinetic properties to the branded drug. These studies are usually performed in 24-36 healthy subjects in whom key pharmacokinetic parameters are analyzed [185-187]. As transplant patients require life-long immunosuppressive treatment, less expensive generic drugs may offer economic advantages. However, in light of the steadily improved survival in the modern immunosuppressive era, it is understandable that clinicians may be reluctant that make such change. Indeed, within the transplant community, the use of generic immunosuppressants has been debated [188-191]. The most important concern is the risk of pharmacokinetic differences between the healthy subjects and the transplant patients, in whom the drugs are tested and used, respectively. In contrast to healthy subjects, transplant patients are not infrequently affected by comorbidities. They are also at risk of drug-drug interactions as they take multiple medications at the same time. Both these factors may influence pharmacokinetics, and thereby safety and efficacy, in transplant patients. With the introduction of Myfenax Teva® and Tacrolimus Sandoz®, it was established in heathy subjects that the two drugs were bioequivalent to CellCept® and Prograf®, respectively. Studies on safety and efficacy in transplant patients, and especially HT patients, are however still few in number. This is also true for other generic versions of MMF and TAC currently available [192].

Paper **IV** indicated that switching from CellCept® to Myfenax Teva® and/or Prograf® to Tacrolimus Sandoz® several years post-HT was safe at least in the short-term perspective (six month period), without detectable changes in TAC C0-levels, safety or efficacy. The findings correspond well with the few earlier studies where safety and efficacy have been studied in transplant patients switching from CellCept® to Myfenax Teva® [193-194] or Prograf® to Tacrolimus Sandoz® [195-201]. However, these studies and other on other generic formulations of MMF and TAC have focused almost entirely on kidney or liver transplant patients. This may be the first study of any generic MMF and the third study of any generic TAC in HT patients [195,202]. It may also be the first time both drugs have been studied simultaneously.

It is important to point out that ~25% of the EMBs obtained during the acute monitoring of the conversion to Myfenax Teva® showed ACR grade 1R. Two deaths also followed this switch. The rate of ACR was however similar in the study and control group, and both paper **I** and **II** indicated relatively high rates of low-grade

ACRs on routine EMBs. It is also unlikely that the deaths were related in any way to the switch. Pulmonary fibrosis is not a commonly observed side effect of MMF - and although it cannot be entirely excluded that the second patient died of rejection - neither the two EMBs nor the autopsy confirmed this diagnosis. Additionally, no clinical improvement was observed despite relatively aggressive treatment.

Paper **IV** was as paper **I-III** also limited by its retrospective design and the relatively few patients included, of which both may have made it difficult to detect significant differences in some of the parameters investigated. The study would have benefited from a longer follow-up and from data on infections, malignancies, AMR, CAV, and costs. Such data were however not available. Moreover, the findings of a cost analysis may not be universally applicable given considerable price variations in different countries. Nonetheless, given the mentioned limitations, the results should be interpreted with caution.

Conclusions

The present thesis, focusing on the HT population at SUS-Lund 1988-2010, provides a useful in-depth overview on the incidence, predictors and outcome of two of the most relevant issues related to the difficult balance between under- and over-immunosuppression after HT – namely ACR and CKD. The main conclusions with particular clinical relevance are:

● The frequency and severity of ACR was low and decreased with time post-HT. The results however indicated that late more often than early ACR remains undetected, and that both types of ACR influence outcome. CKD, which was common and suspected to have been underestimated in earlier studies, also appeared to have a negative impact on survival. Early identification of ACR – perhaps particularly challenging late after HT – and CKD, therefore seems important.

● Heightened attention, with a lower threshold for ACI EMBs early after HT and extended EMB surveillance at later stages (e.g. > 12 months), seems reasonable among "high-risk" patients of ACR, partly defined in paper **I** and **II**.

● Similarly, patients with high risk of rapid GFR loss - partly defined in paper **III** – would likely benefit from closer monitoring of renal function. As the findings indicated that common GFR estimating equations overestimate GFR in HT patients, direct measurements – such as the iohexol clearance method – should be preferred until this has been further studied.

● Although not specifically analyzed, paper **I-III** together give reason to believe that the excellent 10-year survival of 74% vs. 53% in the ISHLT registry – at least to a small extent – may be explained by the frequently performed EMBs and yearly iohexol clearance measurements as opposed to GFR estimating equations, allowing early detection and prevention of both ACR and CKD.

Finally, the debated clinical issue of switching to generic immunosuppressants was evaluated in paper **IV**. Reassuringly, switching from CellCept® to Myfenax Teva® and/or Prograf® to Tacrolimus Sandoz® several years post-HT appeared safe, at least in the short-term perspective. Further studies are however encouraged.

Future perspectives

As long as HT continues to constitute the ultimate treatment choice for end-stage heart failure, and no post-transplant treatment modality superior to immunosuppression exists, issues related to over- and under-immunosuppression will remain a major concern. Tailored management based on each individual patient, likely represents the best approach to improve outcome. Already have there been several strategies developed aimed at minimizing the use of CSs and CNIs. Two examples are the use of induction therapy and mTOR inhibitors, of which the latter have shown promising in preventing CKD and CAV. Also in recent years, several non-invasive genomic methods for rejection surveillance have emerged. Notably, gene expression profiling (AlloMap®) has shown able to reliable rule out severe ACR in asymptomatic low-risk patients later than six months post-HT [203-205], and possibly even earlier than that [206]. Future studies not only need to help improve diagnostics and therapeutics, but also better identify patients at specific risks in order to determine which patients are best suited for a certain strategy. In combination with diagnostic and therapeutic advances, such personalized follow-up programs can hopefully lead to even higher survival rates.

Summary in Swedish (Sammanfattning på svenska)

Bakgrund och syfte

Hjärttransplantation (HT) utgör det sista behandlingsalternativet vid avancerad hjärtsvikt. Efter HT behövs livslång behandling med läkemedel som dämpar kroppens immunförsvar, också kallad immunosuppressiv behandling. Ges inte denna stöts det donerade hjärtat bort och patienten riskerar att dö. Å andra sidan är den immunosuppressiva behandlingen förknippad med flera allvarliga biverkningar. Trots att överlevnaden efter HT stadigt ökat kvarstår problem relaterade till ”under- och överimmunosuppression”, vilka i sig fortsatt utgör några av de viktigaste begränsande faktorerna för långtidsöverlevnaden. Denna viktiga balansgång är också en stor anledning till debatten om huruvida nytillgänglig generisk immunosuppression, som kan vara ekonomiskt fördelaktig, kan introduceras.

HT har utförts vid Skånes universitetssjukhus i Lund (SUS-Lund) sedan 1988. Sedan starten har patienter som genomgått HT även genomgått ett extensivt uppföljningsprogram, bland annat inkluderande njurfunktionsmätningar med hjälp av den så kallade iohexolclearancemetoden samt hjärtbiopsier för att diagnostisera så kallad akut cellulär avstötning (ACR).

Denna avhandling består av fyra arbeten. Arbete **I-III** syftade till att studera förekomst, riskfaktorer och prognos vad gäller ACR och kronisk njursvikt (CKD) – två av de mest relevanta problemen relaterade till balansen mellan under- och överimmunosuppression. Ett ytterligare mål var att, bland patienter som genomgått HT, utvärdera tillförlitligheten av två rekommenderade metoder för att estimera njurfunktion (CKD-EPI och Schwartz). Arbete **IV** syftade istället till att undersöka säkerhets- och verkansutfall efter byte till två generiska immunosuppressiva läkemedel, nämligen Myfenax Teva® och Tacrolimus Sandoz®.

Metoder

Data insamlades retrospektivt och analyserades därefter. Arbete **I-III** baserades på alla 215 patienter som följts i Lund 1988-2010, medan arbete **IV** inbegrep en mindre grupp på 55 patienter.

Resultat

Frekvensen och allvarlighetsgraden av ACR var låg och minskade med tid efter HT. Resultaten indikerade dock att sena (>1 år) i högre utsträckning än tidiga (<1 år) ACR förblev oupptäckta, samt att båda typer av avstötningar är prognostiskt ogynnsamma. Vidare var CDK vanligt samt föreföll påverka överlevnaden negativt. Intressant nog indikerade resultaten också att CKD-EPI och Schwartz båda överskattar njurfunktion hos patienter som genomgått HT, vilket i sin tur skulle kunna leda till diagnostisk fördröjning av CKD såtillvida dessa metoder helt förlitades på. I arbete **IV** indikerade resultaten att bytet till Myfenax Teva® och/eller Tacrolimus Sandoz® flera år efter genomgången HT var säkert, åtminstone ur ett kortsiktigt perspektiv.

Slutsats

I denna avhandling, med fokus på HT-populationen vid SUS-Lund 1988-2010, redogörs en användbar detaljerad översikt av förekomst, riskfaktorer och prognos vad gäller två av de mest relevanta problemen relaterade till den svåra balansen mellan under- och överimmunosuppression efter HT - nämligen ACR och CKD. Utöver detta presenteras värdefull tidig erfarenhet av byte till generika.

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Appendix

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The balance between under- and over-immunosuppression after heart transplantation with emphasis on acute cellular rejection and chronic kidney disease

Experiences from Skåne University Hospital in Lund 1988-2010

Carl Söderlund was born in 1989 in Stockholm, where he also grew up. He moved to Lund in 2009 to study medicine at Lund University, and two years thereafter he got engaged in research within the field of heart transplantation at the cardiology department. He obtained his medical degree in 2015 (picture above) and then moved back to his home town, where he is now working.

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