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Immunosuppression with Everolimus in Paediatric Heart Transplantation

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Contents

Abstract

Background

So far no experience exists on the use of everolimus as de novo therapy in paediatric cardiac transplantation. This is the first and only report on everolimus in paediatric cardic transplantation.

Methods

Fifty-two paediatric heart transplant recipients everolimus (n=24) or MMF (n=28) in combination with CsA as de novo immunosuppresive therapy were investigated. The main study variables were efficacy of everolimus, survival and safety. Further, CsA trough levels, BPAR, laboratory values and angiography evidence of CAV were studied in a follow-up period of 24 months.

Results

One child in the everolimus group and 5 in the MMF group died (p=0.200). Mean daily dose of everolimus was 1.16 $\pm$0.56 mg/qm body surface area resulting in trough levels after 1 week of 5.08 $\pm$2.42 ng/ml and of 4.8 $\pm$1.3 ng/ml at the end of the follow-up period. CsA trough levels in the everolimus group were 167.1 ng/ml vs. 283.8 ng/ml in the MMF group at 6 months post-transplant, 134.1 $\pm$ 50.7 ng/ml vs. 234.9$\pm$23.8 ng/ml (12 month) and 79.3 ng/ml vs. 76.0 ng/ml (24 month). Two children died due to acute cellular rejection in MMF group. Further two cases out of each group experienced BPAR ISHLT grade $\geq$ 3A (p=1.000). Creatinine levels increased in both groups in the first three weeks (1.42 mg/dl vs. 0.48 mg/dl) returning to normal values after 24 months (1.1mg/dl vs. 0.99 mg/dl). 4 cases in everolimus group and 5 cases in MMF group developed CAV. CMV infection were one case in everolimus group, four in MMF group. Side effects such as infections, hospilitation rates, hemoglobin concentration, platelet count, the development of lymphproliferative disease and cholesterol values were similar between the groups.

Conclusion

Everolimus with reduced CsA trough level regime in a paediatric population was safe, effective and comparable to other immunosuppression regimes. It may confer more potential benefits especially on CAV, CMV infections and malignancies.

Zusammenfassung

Hintergrund

Derzeit besteht keine oder eine nur geringe Erfahrung mit dem Gebrauch von Everolimus als de-novo Therapie zur Immunsuppression bei Kinderherztransplantationen. Dies ist die erste und unseres Wissens einzige vergleichende Arbeit zu diesem Thema.

Methodik

Zweiundfünfzig pädiatrische Patienten nach Herztransplantation, die Everolimus (n=24) oder MMF (n=28) in Kombination mit CsA als de novo immunsuppressive Therapie erhalten haben, wurden untersucht. Die Hauptstudienvariablen waren Wirkung und Effektivität von Everolimus, Überleben und Sicherheit nach dieser Therapie. Weiterhin wurden Tal-Spiegel von CsA, durch Biopsie bestätigte Abstossungsreaktion, Laborwerte und angiographische Hinweise zum Vorliegen einer Transplantatvaskulopathie in einer Anschlussperiode von 24 Monaten studiert.

Ergebnisse

Ein Kind in der Everolimus-Gruppe und 5 in der MMF-Gruppe sind (p=0.200) verstorben. Die tägliche Mitteldosis von Everolimus betrug 1.16±0.56 mg/qm-Körperfläche, die auf Tal-Spiegel von 5.08±2.42 ng/ml nach 1 Woche und von 4.8±1.3 ng/ml am Ende der Anschlußperiode resultieren. Der Tal-Spiegel von CsA in der Everolimus-Gruppe betrug 167.1 ng/ml entgegen 283.8 ng/ml in der MMF-Gruppe in der postoperativen Phase von 6 Monaten, 134.1±50.7 ng/ml gegen 234.9±23.8 ng/ml (nach 12 Monaten) und 79.3 ng/ml gegen 76.0 ng/ml (nach 24 Monaten). Zwei Kinder starben wegen einer akuten Abstossung in der MMf-Gruppe. Weiter wiesen je zwei Fälle aus jeder Gruppe ein durch Biopsie gesichterte Abstossung nach ISHLT-Grad > 3A (p=1.000) auf. Die Creatinin-Werte haben in beiden Gruppen, in den ersten drei Wochen, (1.42 mg/dl gegen 0.48 mg/dl) zugenommen, zu dann mittlerweile normalen Werten nach 24 Monaten (1.1mg/dl vs. 0.99 mg/dl). 4 Fälle in der Everolimus-Gruppe und 5 Fälle in der MMf-Gruppe haben eine Transplantatvaskulopathie entwickelt. CMV-Infektionen liessen sich ein Fall in der Everolimus-Gruppe, vier in der MMF-Gruppe evaluieren. Nebenwirkungen der Medikation wie Infektionen, zwischenzeitliche Wiederaufnahme ins Krankenhaus aus

verschiedenen Gründen ebenso Hämoglobin-Konzentration, Thrombozyten-Zahl, die Entwicklung der lymphoproliferativer Krankheiten oder der Vergleich der Cholesterin-Werte waren zwischen beiden Gruppen ähnlich.

Fazit

Everolimus in Kombination mit dem reduzierten Tal-Spiegel-Regime von CsA in der untersuchten Gruppe von pädiatrischen Patienten war sicher, wirksam und mit anderen immunsuppressiven Thrapien vergleichbar. Es könnte mehr potenzielle Vorteile besonders in Bezug auf die Entwicklung von Transplantatvaskulopathie, CMV-Infektionen und der Entwicklung bösartiger Erkrankungen bieten.

Abbreviations and Acronyms

ISHLT	International Society for Heart and Lung Transplantation
CAV	cardiac allograft vasculopathy
CNI	calcineurin inhibitor
FDA	Food and Drug Administration
C_0	basal concentrations
CsA	cyclosporine A
C_2	concentration 2 hours post-dose
IVUS	intravascular ultrasound
AZA	azathioprine
MMF	mycophenolate mofetil
MPA	mycophenolic acid
CMV	cytomegaoviyns
PSI	proliferation-signal-inhibitors
IL-2	interleukin-2
IL-15	interleukin-15
mTOR	mammalian target of rapamycin
IMEG	intramyocardial electrocardiogram
TDE	tissue Doppler echocardiography
BPAR	biopsy proven acute rejection
GFR	glomerular filtration rate

1 Introduction

1.1 Heart transplantation

1.1.1 General history

Organ transplantation is one of the legends in both Eastern and Western cultures. The work of Lieh Tzu in 300 B.C. reports the use of a "wonderful drug" by the noted Chinese physician Pian Ch'iao to promote the survival of heart allografts exchanged between two patients [1]. Dr. Christiaan Barnard performed the first human heart transplant on December 3rd, 1967 in Cape Town, South Africa. After that, the procedure was discontinued in many centres for several years because of an unacceptably high risk of complications concerning the available immunosuppressive agents and failure to control acute rejection episodes.

Effective immunosuppression is essential for transplantation. Since the early 1980s, after the introduction of cyclosporine, which diminished acute rejection, the number of heart transplants worldwide increased significantly to reach the high limit of about 4,500 cases in 1994. Since then, the number reported to the International Society for Heart and Lung Transplant (ISHLT) registry each year has continued to decrease, showing 3,122 heart transplants reported in 2001.

1.1.2 Challenges for heart transplantation

Nevertheless the development of transplantation techniques worldwide has intensified many critical concerns. One of transplantation's greatest barriers is the chronic shortage of donor organs. This is mainly because of the ambiguous feelings towards organ donation and a shortage of cardiac allograft for emergent needs. Supply has not kept pace with demand. Over the past 5 years the shortfall has become worse. No country has sufficient organs to satisfy its citizen's needs except Belgium, Austria, and Spain. The scarcity affects countries worldwide including the United States despite a well-organized national distribution system. Patient survival has improved somewhat over the years. Overall 1-year survival of adult patients after transplantation is about 83% and 3-year survival is about 75%[2]. However, ensuring long-term survival of transplanted organs remains a challenge for transplant clinicians because the shortage of donor

organs and failure of cardiac grafts generally result in death, as re-transplantation is often impossible. The rate of cardiac graft failure 10 years post-transplant is in the order of 50% [3] compared to about 35% for renal transplant recipients.

Postoperative complications such as infections, rejection episodes, side effects of immunosuppressive agents, cardiac allograft vasculopathy (CAV) and neoplasms remain frequent in the early and late follow-up period after heart transplantation.

With respect to causes of death, non-specific graft failure is responsible for 41% of deaths during the first 30 days post-transplant. During the first year non-cytomegalovirus infection is the primary cause (35%). However, acute rejection causes only 12% of deaths 1 year after cardiac transplantation. CAV is the primary cause of late morbidity and mortality in many recipients and represents 17% of annual mortality beyond 3 years after transplantation [4]. After 5 years CAV and late graft failure (31% together), malignancy (24%) and non-cytomegalovirus infection (10%) are the most prominent causes of death [5].

1.2 Immunosuppression in heart transplantation

Clinical organ transplantation has captured the public imagination like no other recent development in surgery. Multitude disciplines were utilized in biology, immunology and pharmacology to improve our understanding regarding the responses of the human body to the transplanted organ.

The advent of effective pharmacologic immunosuppression is a milestone in this field. Immunosuppressive regimens routinely contain a calcineurin inhibitor (CNI), an adjunct immunosuppressant (e.g. azathioprine, mycophenolate mofetil or everolimus) and corticosteroids with or without antibodies. This combination has effectively reduced the risk of early graft failure due to acute rejection in heart transplant recipients.

1.2.1 The efficacy and side effects of CNIs

T cells have been identified as the cells responsible for the initiation of destructive immune responses targeted against allogenic tissue grafts.

Cyclosporine, the first developed CNI, was found to have immunosuppressive properties in 1972, and was approved by the Food and Drug Administration (FDA) in the United States for clinical use in 1983, since when it has revolutionized the field of clinical transplantation. The advantage offered by CNIs compared to the cytotoxic immunosuppressant is their specific action on the immune system without affecting other rapidly proliferating cells. Their main mechanism of action involves binding to specific proteins to form complexes that block the action of calcineurin, a key participant in T-cell activation, thereby blocking the signal transduction pathway responsible for T-cell and B-cell activation [6].

Tacrolimus (FK506), the product of Streptomyces tsurubaensis fermentation, another CNI discovered in 1984, is a macrolide antibiotic that inhibits T-cell activation and proliferation as well as production of other cytokines. It was first used in clinical studies in 1988 at the University of Pittsburgh [7-13].

Several prospective studies have confirmed that cyclosporine (conventional formulation) and tacrolimus have a similar efficacy in preventing acute rejection of heart transplants and are associated with similar patient survival for up to 5 years [14-18]. These studies determined cyclosporine exposure using basal concentrations (C_0) and the whole blood cyclosporine A (CsA) concentration when measured by sampling immediately before the next dose was given. C_0 has been used to monitor exposure to CsA and to facilitate rapid determination of appropriate dosing regimens. This applies particularly to immunosuppressive drugs, most of which have a very narrow therapeutic index so that optimal dosing in the individual patient can only be achieved by therapeutic drug monitoring as an essential component of then long-term management to achieve an optimum balance between adequate immunosuppression and toxic side effects.

However, it is now known that cyclosporine concentration 2 hours post-dose (C_2) is an accurate predictor of total cyclosporine exposure [19]. The C_2 paradigm has been introduced into liver and renal transplantation successfully where it correlates with improved clinical outcomes [20]. The

clinical benefits of C_2-based monitoring also appear to be promising in heart transplant recipients although it remains to be demonstrated whether the short or long-term efficacy of cyclosporine in heart transplantation will be further improved by using C_2 monitoring [21].

The introduction of cyclosporine into clinical practice has stimulated transplantation worldwide. However, cyclosporine is associated with several adverse side effects such as hypertension, hyperlipidaemia and nephropathy and little has been gained with regard to the most important problem: the prevention of CAV. There is now considerable evidence that CNIs may only partly inhibit calcineurin at clinical doses and that this partial inhibition may initiate the immune rejection process leading to CAV [22, 23]. So far, studies of CAV in heart transplant recipients have been restricted by the insensitivity of coronary angiography to detect this condition [24]. Even by this method CAV is present in 42% of patients after 5 years of follow-up [25].

The technique of intravascular ultrasound (IVUS) is more sensitive than angiography and detects the presence of atherosclerosis in patients showing no angiographic evidence of coronary disease. In a study of 262 heart transplant recipients, all patients younger than 30 years of age had an unobtrusive angiogram but IVUS showed that 28% already had evidence of atherosclerosis [26]. CAV may happen at any time, but events during the first year after transplantation seem to be important in its pathogenesis. Progression of intimal thickening of ≥ 0.5 mm in the first year after transplantation is a reliable alternative marker for subsequent development of CAV and so for mortality after heart transplantation [27].

Today, late graft loss due to CAV remains a major challenge for transplant teams. It affects up to 50% of all heart transplant recipients within the first 5 years of surgery [28] although intimal thickening may be present in up to 58% of patients 1 year after transplantation [29].

Currently, the mechanisms of CAV are not fully understood. CAV is a complex, multi-factorial process arising from both immunogenic and non-immunogenic mechanisms [30]. Factors relating to the transplant procedure itself (e.g., ischemia or reperfusion damage) seem to be among the most important non-immunogenic factors of CAV, because they lead to endothelial cell injury. Cardiovascular risk factors (e.g., donor age, hypertension, hyperlipidaemia, and diabetes),

immunologic risks (e.g., acute rejection episodes, anti-HLA antibodies), the side-effects of immunosuppression (CNIs, corticosteroids) and anti-endothelial antibodies after cytomegalovirus infection or nephrotoxicity have all been involved in the development of CAV. Early immunologic and non-immunologic endothelial damage may initiate pathologic remodelling, resulting in progressive luminal narrowing that is characterized by diffuse, concentric thickening of the coronary arteries, in particular due to proliferation of smooth muscles cells in the media[31]. This leads to luminal stenosis throughout the coronary tree and possibly to occlusion of small vessels. Eventually the blood supply of the graft is compromised. CAV can begin soon after transplantation, developing rapidly in a matter of months or years and result in graft ischemia and graft failure. The pathology differs with the coronary artery disease in the general population where atherosclerotic, lipid-filled plaques form at discrete locations in the major coronary arteries. CAV often progresses silently without chest pain but the first presentation may be a major cardiac event such as ventricular arrhythmia or heart failure. So far CAV seems to be not reversible.

Thus, the diagnosis of CAV and monitoring of its progression is very important. Conventional treatments such as angioplasty or coronary bypass only provide a palliative approach. Re-transplantation is a good but rarely performed option because of the so far poor outcomes after surgery and the shortage of donor organs. Therefore, preventing and/or slowing down the progression of vascular remodelling seem to be the most efficient approach.

CNIs effectively prevent acute allograft rejection and so reduce the subsequent risk of CAV, as immunologic risk factors for CAV are acute rejection, humoral rejection and antibody synthesis. However, CNIs are associated with side effects such as hyperlipidaemia, hypertension and new-onset diabetes after transplantation that can contribute to the development of CAV.

The two main approaches to the prevention of CAV are modification of existing risk factors (e.g. anti-hypertensive and lipid-lowering treatment) and optimizing the immunosuppressive regimen in terms of choosing the appropriate CNI to minimize cardiovascular risk as well as reduction of CNI dosage by effective monitoring and appropriate management of CNI levels.

It is not clear at present whether there are any differences between cyclosporine and tacrolimus in effectiveness of preventing long-term development of CAV. The incidence of CAV using tacrolimus when compared to cyclosporine is variously reported [17, 23,24].

Owing to the benefits of CNIs, the survival rates after heart transplantation markedly improved. However, with the increase in use, the side effects of this drug group became more evident [32].

Impairment of renal function is one of the major side effects associated with CNI-based immunosuppression and is similar with both cyclosporine and tacrolimus [16, 28]. However, it is well known that CNIs' nephrotoxicity may be reversible[33]. Chronic renal failure is one cause of morbidity after cardiac transplantation: 5% to 10% of all annual cardiac-transplant recipients worldwide (3,000 to 4,000) develop end-stage renal failure and undergo chronic haemodialysis with reduced quality of life and decreased life expectancy [34-36]. The main factors triggering the development of renal failure are chronic preoperative renal hypoperfusion caused by heart failure and post-transplant nephrotoxicity of CNIs [37].

CNIs cause specific alterations of the afferent arterioles and less specific glomerulum sclerosis, tubular atrophy, and interstitial fibrosis in the heart transplant patients. These agents play a key role in the development of chronic renal impairment [38].

Therefore therapeutic strategies have to focus on alternative and less nephrotoxic immunosuppressive medications.

The incidence of most types of infection is similar in heart transplant recipients treated with cyclosporine and tacrolimus[15-18] , although severe infections may occur significantly more often in those treated with tacrolimus[39, 40]. This may reflect differences in dosage patterns and the degree of overall immunosuppression achieved.

Furthermore, the incidence of tremor and cosmetic side effects (hirsutism and gingival hyperplasia) are common with CNI immunosuppression [7].

One of the most important predictors of patient mortality at more than 5 years after heart transplantation is CAV. Neither cyclosporine nor tacrolimus have been shown to prevent the development of CAV.

After renal transplantation, tacrolimus is more likely than cyclosporine to cause impaired glucose tolerance [41, 42] and development of new-onset diabetes [17]. Concerning heart transplantation some studies have shown a similar trend [15-17, 43-45]. Several studies have suggested that new-onset diabetes increases the risk of cardiovascular death (cardiac, cerebral and peripheral) and other adverse outcomes in heart transplantation [40, 46-48].

The cardiovascular side effects of CNIs may be exacerbated by the concomitant use of corticosteroids, so minimization of corticosteroid dosage, or discontinuation of steroids in selected patients, may help to attenuate these effects [49- 51].

Although CNIs show a low immunosuppressive hazard for infection and malignancy, these drugs produce varying degrees of nephrotoxicity, neurotoxicity and glucose intolerance. To minimize the adverse side effects of CNIs, one approach is to develop an immunosuppressive protocol combining drugs that act synergistically to offer high efficacy against rejection while minimizing the toxic side effects.

1.2.2 Adjunctive immunosuppressants in heart transplantation

Earlier clinical development of immunosuppressive agents focused on the prevention of acute rejection because rejection and complications of rejection therapy remain the primary causes of death in the first year after heart transplantation.

Azathioprine (AZA) and steroids were among the first drugs available for pharmacologic immunosuppression. From 1966 to 1983, the AZA/prednisone regimen was considered the "conventional" immunosuppressive protocol in solid organ transplantation. Both suppress lymphocyte proliferation and lymphocyte function. However both drugs show severe dose-related side-effects such as bone marrow aplasia, gastrointestinal visceral perforation and overwhelming sepsis when used over longer periods of time.

Now, transplantation has moved from the era of non-selective antiproliferative treatment towards the era of selective T cell depression using CNIs and drug synergy. Adjunctive immunosuppressants complement the action of the CNIs. The standard adjunct to cyclosporine has been AZA, an inhibitor of purine biosynthesis.

Mycophenolate mofetil (MMF), a synthetic analogue of mycophenolic acid (MPA) inhibits the de novo purine synthesis pathway in lymphocytes. In 1995, it was approved by the FDA in the United States and was introduced in renal transplant patients as a replacement for AZA in an effort to further reduce both acute and chronic rejection as well as adverse side effects. MPA, the active metabolite of MMF, selectively inhibits lymphocyte proliferation by blocking the de novo synthesis of guanosine nucleosides. A 3-year, double-blind, randomized comparison of these agents in combination with cyclosporine and prednisone showed that 1-year mortality was significantly lower in patients treated with MMF than among those treated with AZA, and the incidence and severity of rejection were reduced[52]. The survival advantage with MMF was attributed to reduced cardiovascular deaths overall [53]. In terms of the efficacy in preventing CAV, MMF provides a modest advantage over AZA, and the combination of cyclosporine plus MMF results in significantly lower mortality than cyclosporine plus AZA. Although MMF is substantially more expensive than AZA, the reduced costs of treating rejection episodes may compensate for this disadvantage.

Gastrointestinal complaints (type B symptoms) and haematological disorders (leucopoenia, anaemia, and thrombocytopenia) accounted for the most common adverse side-effects, resolving after drug discontinuation.

There was a dose-dependent trend towards more opportunistic infections: tissue invasive cytomegalovirus (CMV) infection occurred more frequently in the MMF 3g/day group, but uniform prophylaxis was not required.

The proliferation signal inhibitors (PSI) show no nephrotoxicity in wholesome kidneys, but have potential to facilitate reduced CNI dosing. The PSI class consists of everolimus (Certican®, Novartis Pharma AG, Basel, Switzerland) and sirolimus (Rapamune®, Wyeth Pharmaceuticals, USA).

Rapamycin (sirolimus), a macro cyclic immunosuppressive agent and product of streptomycin hygroscopicus relies on inhibition of the interleukin-2 (IL-2) mediated signal transduction pathway, thus targeting a later stage in the cascade of lymphocyte proliferation.

The combination of rapamycin and CNIs shows a notable reduction in acute rejection episodes compared to CNIs alone. Adverse side effects were mainly attributed to laboratory abnormalities such as hyperlipidaemia and haematological changes like leucopoenia and thrombocytopenia. Renal function parameters are favourable, and there was less hypertension detectable. Owing to the synergism with cyclosporine, it is therefore likely that rapamycin may facilitate dosage reduction of both CNIs and corticosteroids, thus reducing toxic side effects in maintenance of immunosuppressive patients.

In addition to its effects on immune cells, rapamycin also reduced proliferation of vascular smooth muscle cells [54]. Rapamycin class compounds therefore showed considerable potential for reducing the incidence and progression of CAV.

1.2.3 Everolimus, a new adjunctive immunosuppressant

The immunosuppressant everolimus, a new derivative of rapamycin, a PSI, has recently been introduced into cardiac transplantation and has similar mechanisms of action as sirolimus [55].

Everolimus is a novel inhibitor of the p70 S6 kinase, which arrests the cell cycle of lymphocytes and vascular smooth muscle cells in the G_1 phase. It also has immunosuppressive effects via inhibition of T-cell and B-cell proliferation mediated by IL-2 and interleukin-15 (IL-15). The cytochrome P450 enzymes 3A4, 3A5, and 2C8 are the major sites of everolimus metabolism so the drug moderates the immune and non-immune response to the allograft and reduces the risk of acute rejection and CAV [9]. In addition everolimus inhibits growth-factor-dependent proliferation of cells through a calcium-independent signal [55, 56], whereas CsA inhibits T-cell-dependent growth factors through a calcium-dependent signal. The synergistic immunosuppressive effect of everolimus combined with cyclosporine and methylprednisolone significantly reduces T-cell proliferation and allows the reduction of CsA dosage [57].
This may have the dual benefit of reducing risk of nephrotoxicity and CAV without any added risks of acute rejection that have been reported in animal models and in renal studies [55, 58].
Therapeutic drug monitoring studies in both renal and heart transplantation have shown that everolimus at blood levels of 3 to 8 ng/ml were associated with a 2.5-fold lower rate of rejection than levels < 3ng/ml ($p < 0.0001$), while at blood levels of > 8ng/ml it did not confer any extra efficacy benefit [50, 51]. Therefore it is believed that, before reducing CsA blood levels, everolimus at levels of 3 to 8 ng/mL should be achieved. Usually steady blood levels within the desired range using concentration-controlled everolimus to minimize CsA exposure could be achieved after 1 to 2 weeks of initiating everolimus [39]. A 25% to 30% reduction in CsA blood levels for patients receiving everolimus was thought reasonable without compromising immunosuppressive
efficacy [41]. Everolimus and basiliximab therapy in renal transplant recipients meant that CsA exposure could be reduced by approximately 50% during the months after transplantation [53].
The combination of these two approaches could be an appropriate course of treatment for heart transplant patients with preoperative renal dysfunction [41]. Everolimus can decrease vascular remodelling by preventing growth-factor-mediated smooth muscle cell proliferation and attenuating vascular neointimal formation in animal models[55]. The potential for reducing intimal hyperplasia in coronary arteries in animal models of immune and non-immune injury is

also being realized in humans[45]. Chronic allograft dysfunction is the major barrier to long-term graft and patient survival after transplantation [59, 60]. Everolimus prevents vascular remodelling and neointimal proliferation, which are both key components of CAV, as well as suppressing T cells. Data from drug-eluting stents (sirolimus and everolimus) also demonstrated the efficacy of PSI for preventing or slowing pathological coronary vascular remodelling [61, 62].

As with other immunosuppressive agents, everolimus may increase certain types of infection (e.g. bacterial infections including pneumonia) and increase the blood lipid levels, but they can be managed [24, 40]. The clinical development of this class of compounds is unusual because, for the first time in the history of immunosuppressive agents, a class of compounds has been designed to treat a specific unmet medical need in heart transplantation. Earlier clinical development of immunosuppressive agents focused only on one common theme in all organ transplantation: the prevention of acute rejection [63].

1.2.4 Induction therapy with antibodies

The use of antibodies to build up an effective immunosuppression was started by using polyclonal sera developed in animals such as horses or rabbits. The mechanism by which polyclonal sera cause immunosuppression is not yet well understood, although cell-mediated cytotoxicity of lymphocytes in the circulation may be one major effect. In contrast the monoclonal antibody OKT3 is more specific for the T-cell receptor, thus preventing activation of T-lymphocytes. Most recently the human and chimeric murine monoclonal antibodies daclizumab and basiliximab have provided effective induction therapy with fewer adverse effects.

Nevertheless immunosuppressive medication still displays severe side-effects too often. New immunosuppressive agents have been investigated, seeking to prevent or reduce these complications. The optimal immunosuppressive strategy to prevent rejections as well as CAV without increasing the risk of immunological events is, however, still under investigation.

2 Aim of the study

For children with intractable congenital heart failure, heart transplantation has been the most effective long-term therapy. Worldwide, an average of 87 centers reported 3865 pediatric heart transplant recipients between 2000 and 2008 to the registry of the ISHLT. Immunosuppression reduces the rate of acute rejection with favourable graft and patient survival but is also accompanied with significant comorbidities. In 2008, 98% of patients received CNI (37% CsA), 66% MMF and 68% steroids for maintenance immunosuppression at 1 year follow-up. In the same patients, 23% of children received a combination of CsA and MMF versus 30% with tacrolimus and MMF for immunosuppression. With the availability of the mammalian target-of-rapamycin (mTOR) and PSI, possible new immunosuppression strategies for adult heart transplantation recipients have emerged. In adult heart transplantation recipients treated with CsA, Eisen et al. demonstrated a superior efficacy of everolimus compared to AZA with regard to acute allograft rejection and CAV [45]. In 2004, these study results led to the approval of everolimus for heart transplantation in Europe. FDA approval of everolimus in North America was denied because a significant number of patients encountered renal dysfunction with non-trough-leveled everolimus and combined fixed doses of CsA. A randomized, open-label and prospective multicentre study by Lehmkuhl et al. in adult de-novo heart transplantation recipients demonstrated a safe dose reduction of CsA combined with trough-leveled everolimus (target 3 to 8 ng/ml) resulting in stabilized renal function without loss of efficacy compared to patients receiving standard doses of CsA combined with MMF [64]. Single center experiences with everolimus advocated such a strategy beforehand [65, 66]. Data from adult populations cannot necessarily be transferred and applied to pediatric heart transplantation recipients. To date, one multicentre has demonstrated favorable efficacy and safety of everolimus in a pediatric kidney transplant recipients [67] but no data is available for pediatric de-novo heart transplantation recipients. In analogy to the study in adult heart transplantation recipients [64], the objective of this study was to establish an immunosuppression protocol trough-level-guided everolimus

combined with reduced doses of CsA and determine efficacy and safety compared to MMF and standard doses of CsA in pediatric heart transplantation recipients.

Therefore, we conducted a retrospective analysis by collecting the data of the patients younger than 18 years who were transplanted at the Deutsches Herzzentrum Berlin (German Heart Institute Berlin) between 2000 and 2006. The purpose of the study was to describe the recent 8-year experience with heart transplantation in small children and young adolescents with intractable heart failure, and also a better understanding of treatment with everolimus combined CNIs and steroid protocols in the pediatric heart transplantation population.

3. Patients and methods

3.1 Study population

Between January 1, 2000, and December 31, 2006, 58 patients younger than 18 years old with end-stage congestive heart failure who underwent orthotopic heart transplantation at the Deutsches Herzzentrum Berlin received cyclosporine based triple-therapy immunosuppressive regimens (cyclosporine + steroid + MMF/ everolimus). Twenty-eight of them received MMF, while 24 children were treated with everolimus and another 6 patients were transferred to an everolimus regimen, but all of them were treated with MMF for more than 9 months before conversion to everolimus; the indication for conversion was intolerance of MMF or renal failure. The basic characteristics of the recipients are listed in Table 1.

Table 1. Characteristics of paediatric heart transplant recipients

	MMF group (n=28)	Everolimus group (n=24)
Age (years)	6.9±1.1	11.0±1.2
Female/male	15/13	12/12
Body weight (kgs)	37.3±21.2	31.6±19.9
Height (cms)	139.3±42.4	130.4±37.6
Indication for transplant		
Cardiomyopathy	23	15
Congenital	4	6
Re-Tx	1	3
Waiting time on high urgent status (days)	18.1±5.4 1(0-112)	42.7±7.3 45(0-118)
Waiting time on urgent status (days)	9.3±4.8 0(0-112)	45.1±7.4 47.5(0-133)
Blood group	A12 B3 AB1 O12	A9 B0 AB1 O14
Rh-	4	2
CMV mismatch	4	6
Waiting time (days)	64.9±12.7 49(1-293)	93.3±26.761.5(6-616)
Ischemic time (minutes)	221.9±10.6 224(92-382)	237.9±11.7 235.5(119-356)

3.2 Donor availability

The donor hearts were allocated by the Eurotransplant Organization from Leiden, The Netherlands. Table 2 shows the characteristics of the donors.

Table 2. Heart donor parameters

	MMf group	Everolimus group
Donor age (years)	13.0±2.8	21.0±3.1
Ischemic time (minutes)	213±11.0	237±11.5
Body weight (kgs)	34.4±4.0	50.2±5.1
Height (cms)	128.2±7.4	146.8±7.3
Gender(female/male)	13/16	13/11

Operative technique

All operations were performed using cardiopulmonary bypass. The diseased hearts were resected, leaving behind the posterior walls of the left atrium, superior caval vein and inferior caval vein, the and the stumps of the aorta and pulmonary artery.

The donor hearts were arrested with 2,000 to 3,000 mL of cold HTK cardioplegic solution (Dr. Franz Koehler Chemie GmbH, Germany). The donor heart was explanted by transecting both caval veins and the four pulmonary veins after dividing the ascending aorta and the pulmonary artery, preserving the sinus node, its artery, and the sinoatrial pathways. The donor hearts were stored in cold cardioplegic solution during transportation. Bi-caval technique was used for the implantation procedure: first, the left atrium was anastomosed with continuous 3/0 or 4/0 polypropylene suture, followed by anastomoses of both caval veins with running 4/0 or 5/0 polypropylene suture; later the pulmonary artery and the aorta were anastomosed with 4/0 or 5/0 polypropylene suture continuously. In some patients with congenital heart defects, anatomic modifications were made, for example, in patients with hypoplastic left heart syndrome, deep hypothermia and circulatory arrest were used according to the method of Bailey and associates [68] or an atrial septum was created by modifying the atrial incision on the donor heart. A dual-chamber telemetric pacemaker with two epicardial leads on the right and left ventricles was routinely implanted in the left upper abdominal quadrant below the superficial fascia for postoperative monitoring of acute rejection through measurements of the intramyocardial electrogram (IMEG) amplitude.

3.3 Everolimus laboratory assay

Everolimus C0 was measured just before the next dose administration; the blood samples were obtained and determined by liquid chromatography with mass spectrometry, as described previously [69].

3.4 Immunosuppressive protocol

The immunosuppression regimen was based on a triple-drug combination of CsA, steroids, and MMF/everolimus with induction therapy.

CsA was given at a dose of 4 mg/kg of body weight orally or 1 mg/kg intravenously in children younger than 8 years immediately before transplantation.

No induction MMF was given.

The pre-treatment everolimus was administered orally or through a nasogastric tube at a dose of 0.8 to 1.2 mg/qm body surface area, that is 0.125 mg for infants and children weighing less than 15 kg, 0.25 mg for body weight of 15-20 kg, 0.5 mg for 20-40 kg, and 0.75 mg for body weight greater than 40 kg. Methylprednisolone (Urbason®, Hoechst, Germany) was given intravenously immediately before the aortic cross-clamp was opened at a dose of 67.5 mg for infants of less than 10 kg, 125 mg 10-20 kg, 250 mg for 20- 40 kg, 500 mg for 40- 60 kg, and 1000 mg for body weight greater than 60 kg. All of the patients received postoperative immunosuppression with either MMF (CellCept, Roche) or everolimus together with CsA and oral steroid treatment. Dosing of all immunosuppressive agents was adjusted to maintain the blood trough level and current white blood cell at > 3,000 and platelets > 70.000.

All paediatric heart transplant patients received induction therapy with two cycles of anti-thymoglobin antibodies (A`1TG; Fresenius, Bad Homburg, Germany, or Thymoglobuline, Sangstat, Lyon, France) at a dose of 2.5 mg/kg body weight in combination with intravenous methylprednisolone pulse therapy on post-transplant days 1 and 2.

Within 2 to 5 days, intravenous CsA therapy (1.5- 2 mg/kg) was generally switched to oral administration (4-8 mg/kg/day) and some of the children were given oral CsA (Sandimmun Optoral, or Neoral, Novartis Pharma) by nasogastric tube in the first week. Cyclosporine was

measured in whole blood by a liquid chromatography–mass spectrometric method. The pharmacokinetic parameters of cyclosporine were assessed with C0. Cyclosporine dose adjustments were made according to the target trough-levels shown in Table 3.

Table 3.

C0	MMF group	Everolimus group
Month 1 – 3 postop	200 – 250 ng/ml	200 – 250 ng/ml
Month 3 – 6 postop	200 – 250 ng/ml	150 – 200 ng/ml
Month 6 – 12 postop	150 – 200 ng/ml	125 – 150 ng/ml
> Month 12 postop	125 – 150 ng/ml	80 – 125 ng/ml

Maintenance MMF was given at the dose of 1000 mg, 2 -3 times/day.

Everolimus was initiated at a dose of 0.8 -1.2 mg/qm body surface area in two equal doses every day with weekly monitoring to ensure blood levels were maintained toward the upper end of a 3-8 ng/ml range (defined as appropriate target for everolimus blood levels).

The recommended frequecy of therapeutic drug monitoring is shown in Table 4.

Table 4. Everolimus monitoring

Time post-transplant	Everolimus monitoring
Month 0 – 1 postop	Weekly
Month 2 – 6 postop	Bi - weekly
Month 6 – 12 postop	Monthly
> Month 12 postop	Bi-monthly

The frequency of monitoring should increase until the steady state is reached once the dose is changed. An immunoassay kit, the Certican immunoassay is currently available in Germany; it has demonstrated good accuracy for quantifying everolimus over the range of 2-40ng/ml and its precision ranges from 6% to 11% for everolimus concentrations of 25 to 2.5 ng/ml. The assay can be performed in any clinical laboratory and results are generally available in less than 2 hours.

Steroids were given intravenously 4 to 6 hours after the patients arrived in the ICU at the following doses: 3-10 kg, 67.5 mg methylprednisolone, twice/day, 6 times total; 10-20 kg, 125mg methylprednisolone, twice/day, 6 times total; 20-40 kg, 250 mg methylprednisolone

given once 4-6 hours post operation, then 125 mg every 6 hours, 5 times total; > 40 kg, 500 mg methylprednisolone given once 4-6 hours after the patients arrived in the ICU, then 125 mg every 6 hours, 5 times total.

Prednisolone 1 mg/kg/day was given intravenously in two equal doses thereafter, and then prednisone was given orally when possible, and reduced to the target at 14 days post transplant of 0.5 mg/kg, twice/ day; and 0.3 mg/kg, twice/day by the end of 6 weeks; 0.1 mg/kg, twice daily at the end of 6 months; and so on with reduction to 0.05 mg/kg, twice/day at 1 year postoperation, and then, stopping steroids if possible.

3.5 Surveillance of rejection

All patients were monitored daily by observation of IMEG based on day-by-day changes in the maximal QRS complex amplitude and heart rate for detection of rejection. The IMEG voltage was recorded by a receiver coil taped to the skin above the pacemaker
every night when the patient was asleep, and the signals were transferred to a bedside receiver and then transmitted to the computers in our center by telephone modem for data analysis.

Patients with a substantial voltage drop in the QRS complex amplitude of > 10% over 2 days and an increase of heart rate were suspected of having acute rejection and would be immediately instructed to visit the hospital for further rejection diagnosis.

In the case of rejection, conventional echocardiography examination typically shows: increase of wall thickness, decrease of ejection fraction, new or increasing pericardial effusion. Tissue Doppler echocardiography (TDE) is highly sensitive for diagnosis of acute rejection: a downtrend in the TDE systolic velocity of left and right annular ventricular wall motion was highly suggestive of acute rejection.

The combination of downtrends of IMEG and TDE wall velocities was an indication for endomyocardial biopsy although this is not routinely performed without suspicion of rejection at the Deutsches Herzzentrum Berlin.

3.6 Rejection grading

Pathologists were unaware of the immunosuppressive regimen. Rejection grading was defined according to the ISHLT scale for endomyocardial biopsies, as follows:

No evidence of rejection: ISHLT Grade 0.

Focal perivascular infiltrate without necrosis: ISHLT Grade 1A.

Focal interstitial infiltrate without necrosis: ISHLT Grade 1A.

Diffuse but sparse interstitial infiltrate without necrosis: ISHLT Grade 1B.

Single focus of infiltrate with associated myocyte damage:ISHLT Grade 2.

Two to three foci of infiltrates with associated myocyte damage: ISHLT Grade 2-3A.

Multifocal interstitial infiltrates with associated myocyte damage: ISHLT Grade 3A.

Diffuse inflammatory infiltrates with associated myocyte damage:ISHLT Grade 3B.

Diffuse polymorphous infiltrate with myocyte necrosis, edema, hemorrhage, and/or vasculitis: ISHLT Grade 4.

3.7 Treatment of rejection and steroid tapering

Biopsies with Grades 0, 1A, or 1B were considered negative. Grade 2 rejection was treated only within the first 90 days or if accompanied by hemodynamic compromise. We treated Grades 3A or 3B rejection without hemodynamic compromise with either 3 days of methylprednisolone 1,000 mg i.v., or with prednisone 1 mg/kg orally for 3 days, depending on clinical status and rejection history. Hemodynamically significant rejection was treated with 3 days of methylprednisolone 1000 mg i.v. and with OKT3. After treatment of rejection, we immediately resumed steroids at the previous dose.

3.8 CMV prophylaxis

We determined the type of prophylactic medication for cytomegalovirus (CMV) with a risk assessment. We routinely test patients who undergo cardiac transplant and organ donors for IgG anti-CMV antibodies. Patients who test negative for anti-CMV antibodies are at particular risk for acquiring acute CMV infection if they receive an organ from a CMV-positive donor. These

patients received intravenous ganciclovir for 3 to 5 days and oral ganciclovir (Cytovene) for 6 months.

Patients who were CMV negative and received CMV-negative allografts did not require anti-CMV prophylaxis. Remaining patients received acyclovir 800 mg orally 4 times a day for 6 months.

All patients received anti-hypertensive therapy as well as statins (fluvastatin or pravastatin 5-40mg/day).

The coadministration of drugs that are strong inhibtors or inducers of cytochrome P450 metabolizing enzymes was prohibited.

3.9 Follow-up

All patients underwent a pre-transplantation evaluation at our facilities. The transplant team composed of transplant physicians, cardiologists, surgeons, a pulmonologist, and a psychologist provides or coordinates subsequent periodic medical evaluation and early and long-term post-transplantation patients care. In the early postoperative period, the children were examined every week for 2 months, then every 2 weeks, and then every 3 weeks until 6 months after transplantation. Later, they came to our out-patient clinic at variable intervals depending on the postoperative course. Generally patients were referred to us for further diagnostic and therapeutic management if major complications developed during the follow-up period.

Serial steady-state everolimus and cyclosporine trough concentrations as well as hematology and blood lipids, and also the renal function parameters, were obtained at each scheduled visit post-transplant.

3.10 Statistical analysis

3.10.1 Descriptive statistics

Statistical analysis was performed with SPSS, version 10.0.0 for Windows (SPSS, Inc., Chicago, IL). Data are presented as median and range. Comparisons were performed using Student's *t*-test or the Mann–Whitney *U*-test. Qualitative variables were analyzed by Fisher's exact test. Results

are given as mean values ± standard error of the mean. A p value ≤ 0.05 was considered to indicate statistical significance.

3.10.2 Survival analysis

Survival estimates were based on the Kaplan-Meier method and compared by log-rank statistics. Patient survival was calculated from the day of heart transplantation until death.

4. Results

4.1 Patient characteristics

4.1.1 General characteristics of recipients and donors

Most of our patients came from Germany, some were from eastern Europe or northern Europe as well as from the Middle East and Far East. Sometimes, the following up of children was not done at the Deutsches Herzzentrum Berlin during this ongoing study of 2 years; also the adherence of the children was not as good as in adult patients, especially in the transfer group of 6 children, so the data from this group was not discussed in this paper because the number of patients and the values were too small to draw conclusions.

4.1.2 Recipients' demographics

The baseline characteristics of the recipients are listed in Table 5; the two groups were well matched at baseline for all demographic parameters.

Table 5. Characteristics of heart transplant recipients

	MMF group (n=28)	Everolimus group (n=24)	p
Age (years)	6.9±5.8	11.0±5.8	0.078
Infant	6	2	0.262
Female/male	15/13	12/12	0.299
Body weight (kg)	34.4±21.4	50.2±24.8	0.319
Height (cm)	128.2±39.4	146.8±35.7	0.412
Indication for transplant	28	24	0.110
Cardiomyopathy	23	15	
Congenital	4	6	
Re-HTx	1	3	
Waiting time to HTx (days)	64.9±12.7	93.3±26.7	0.114
Blood group	A12 B3 AB1 O12	A9 B0 AB1 O14	0.354
Rh-	4	2	0.402
CMV mismatch	4	6	0.183
Organ			0.724
Heart	27	22	
Heart and kidney	1	2	

Heart transplantation was preceded by ventricular assist device support in 10 and 8 children in the everolimus and MMF group, respectively (p=0.388). There were patients on BVAD support, 1 on RVAD and 6 on LVAD in the everolimus group, while 2 patients were on BVAD and 6 on LVAD support in the MMF group.

4.1.3 Donors' demographics

Table 6 shows that the basic characteristics of the donors were similar in the two groups except that the donors' body weight in the MMF group was significantly lower (p=0.050).

Table 6. Characteristics of heart transplant donors

	MMF group	Everolimus group	P
Age (years)	13.3±14.8	19.4±15.4	0.150
Female/male	13/15	13/11	0,578
Ischemic time (min)	213.4±56.7	237.1±56.2	0.206
Body weight (kg)	35.3±20.6	48.4±26.4	0.050
Height (cm)	130.8±37.6	142.9±39.0	0.260
Blood group	A15 B2 AB1 O10	A9 B2 AB1 O12	0.809
Rh-	7	2	0.113
CMV_+	14	10	0.548
Dopamine Yes	13	5	0.053
Dobutamine Yes	1	1	0.911
Epinephrine No	28	24	1.00

4.2 Pharmacokinetics

4.2.1 Dose of MMF

The median daily dose of MMF was 0.63 (0.53-0.96) g in the MMF group.

4.2.2 Everolimus Exposure

The mean daily dose of everolimus was 0.045±0.026 mg/kg, 1.16±0.56 mg/qm body surface area. Doses of everolimus were adjusted to maintain blood trough levels within 3-8 ng/ml, especially before reduction of cyclosporine exposure.

The mean trough blood level of everolimus reached 4.53±3.47 ng/mL at the time of transplant. The steady state of everolimus C0 blood levels was reached by the first week post-transplant

(5.08±2.42 ng/ml); only 3(15%) patients had levels less than 3 ng/ml and 1 patient had a concentration of more than 8 ng/mL (11.7 ng/mLl). The pharmacokinetic parameters of everolimus were maintained very stable within the target range of 3 to 8 ng/mL over the 24 months after transplantation.

Table 7. Mean(±SD) blood trough levels of everolimus

	Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
n	13	20	16	20	17	15	14	12	10	12	10	9	8	7	7
Mean	4.53	5.08	4.98	5.19	6.11	6.83	5.39	5.71	4.69	5.91	4.90	5.12	4.02	4.09	4.81
SD	3.470	2.424	2.410	2.721	2.516	2.592	1.620	1.774	1.412	2.510	1.853	1.936	1.111	0.805	1.340

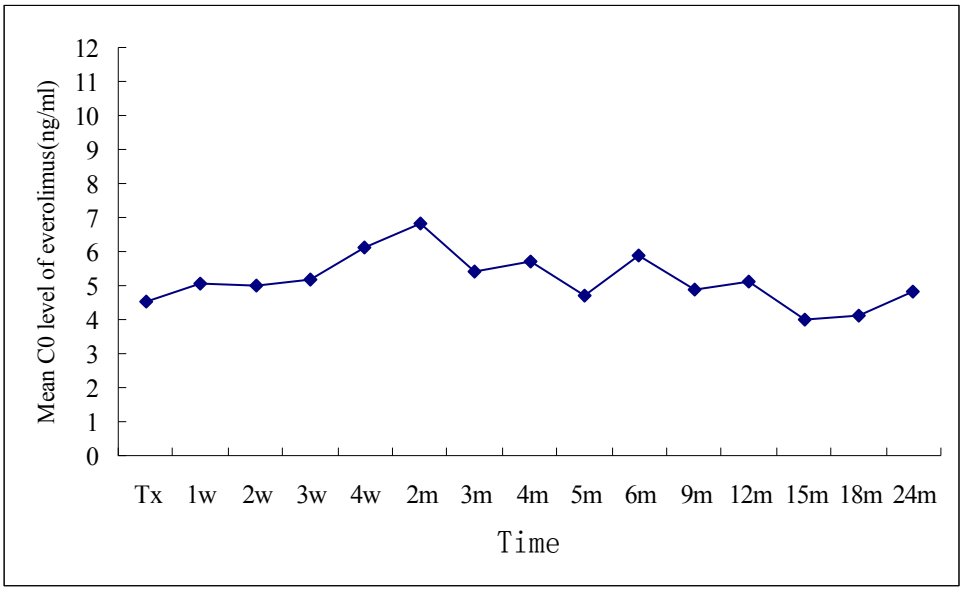

Figure 1. Mean C0 level of everolimus over time

4.2.3 Cyclosporine exposure

Cyclosporine dosage was titrated according to the target trough levels of cyclosporine in both groups.

The trough levels of cyclosporine progressively decreased over time with significant statistical differences between the groups from 2 weeks until 18 months post-transplant.

Everolimus trough levels stablized within the target range of 3-8 ng/ml allowing mean CsA C0 blood levels to be reduced from 217.0±52.2 ng/ml at 2 weeks post-transplant to 167.1±46.6ng/ml

at month 6 and 134.1±50.7 ng/ml by month 12 after transplantation. A decrease in CsA C0 blood levels of 23% by month 6 and a 20% progressive decrease at month 12 was noted.

While CsA C0 blood levels in the MMF group were reduced from 323.5±163.9 ng/ml at 2 weeks after transplantation to 283.8±88.0 ng/ml at month 6 and 234.9±145.9 ng/ml at month 12 post-transplant, a decrease of 12% and progessive decrease of 17% at months 6 and 12 were observed. The trough concentration of cyclosporine for patients receiving everolimus was markedly reduced by 23% to 53% compared to that in the MMF group.

Everolimus C0 blood levels remaining stable within the range of 3-8 ng/ml allows a pronounced reduction in the cyclosporine C0 target levels to less than 250 ng/ml in the 1st month post-transplant compared with 272.8-327.8±129.9 ng/ml within the first month in the full dose CsA regimen. The trough levels of CsA were reduced aggressively to around 100 ng/ml after 12 months post-transplant in the everolimus group.

Table 8. Mean (±SD) blood levels of cyclosporine (C0)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	24	23	23	23	21	19	19	15	11	14	13	10	9	7	7
	Mean	189.3	246.3	217.0	246.3	229.2	222.3	201.8	170.7	164.6	167.1	140.3	134.1	109.3	94.0	79.3
	SD	91.2	98.9	52.2	52.7	70.7	72.7	65.4	35.9	25.1	46.6	47.7	50.7	61.5	16.5	23.8
MMF Group	*n*	23	24	24	25	25	24	20	20	17	16	16	19	12	16	2
	Mean	182.3	272.8	323.5	327.8	321.2	289.7	281.0	294.6	260.1	283.8	249.2	234.9	185.2	201.1	76.0
	SD	131.4	128.7	163.9	129.9	134.1	79.0	75.7	107.7	99.4	88.0	53.1	145.9	41.5	68.0	12.7
	t	0.214	0.789	3.027	2.891	2.826	2.874	3.498	4.798	3.786	4.438	5.740	2.102	3.380	5.915	0.182
	P	0.832	0.434	0.005	0.007	0.007	0.006	0.001	0.000	0.001	0.000	0.000	0.045	0.003	0.000	0.861

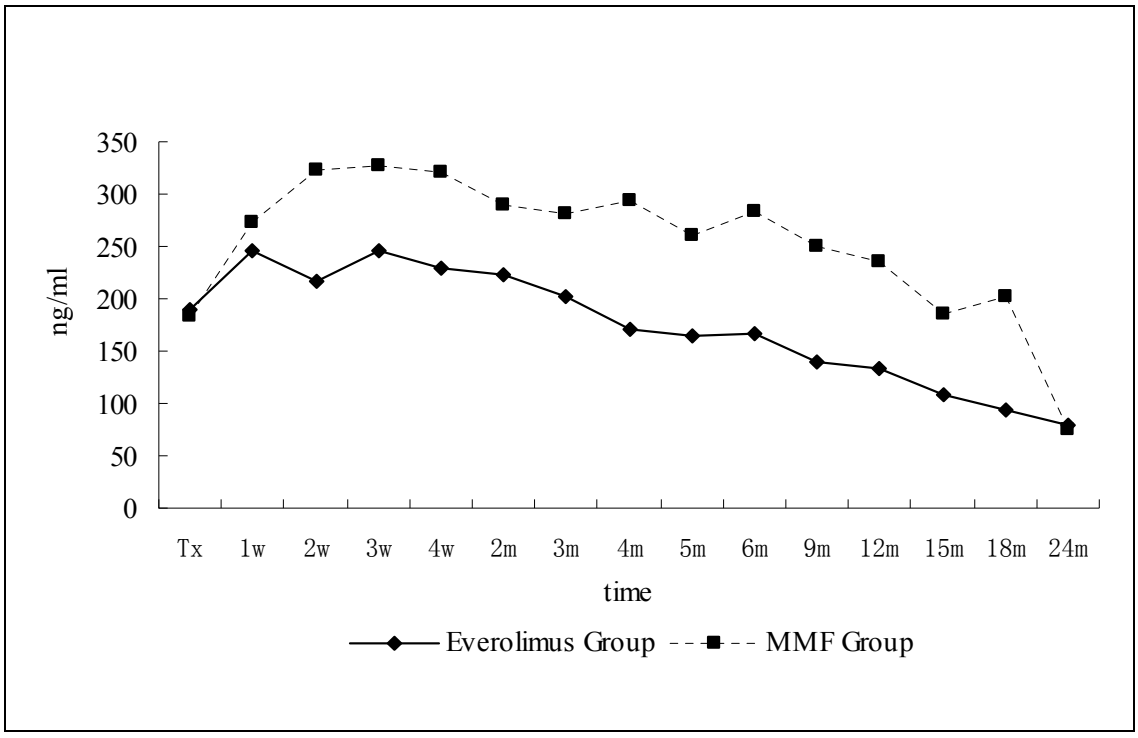

Figure 2. Mean C0 of cyclosporine over time

In the everolimus group, from 2 weeks post-transplant, the blood level of cyclosporine was decreased significantly by month 6 (*P*=0.005), and month 12 post-transplant (*P*=0.000).

In the MMF group, the difference in cyclosporine C0 levels was not significant between week 2 and month 6 (*P*=0.239） post-transplant，but by month 12 post transplantation, a significant decrease was noted (*P*=0.009).

4.3 Efficacy

4.3.1 Effect on survival

There was 1 death in the everolimus group and 5 deaths in the MMF group by 2 years (p=0.200). Survival rate were 0.96 and 0.86 in the everolimus group and MMF group respectively by 2 years. The death in the everolimus group occurred on day 109 due to infection and sepsis. In the MMF group, one death occurred on the 1st post-transplant day due to primary graft failure, another 2 patients died on day 4 and day 32, respectively, because of acute rejection; there was one sudden death on day 46 that was attributed to cardiac arrest during right ventricle biopsy,

and in the 21st month another patient died from ARDS and multi-organ failure. No deaths occured as a result of cardiac allograft vasculopathy.

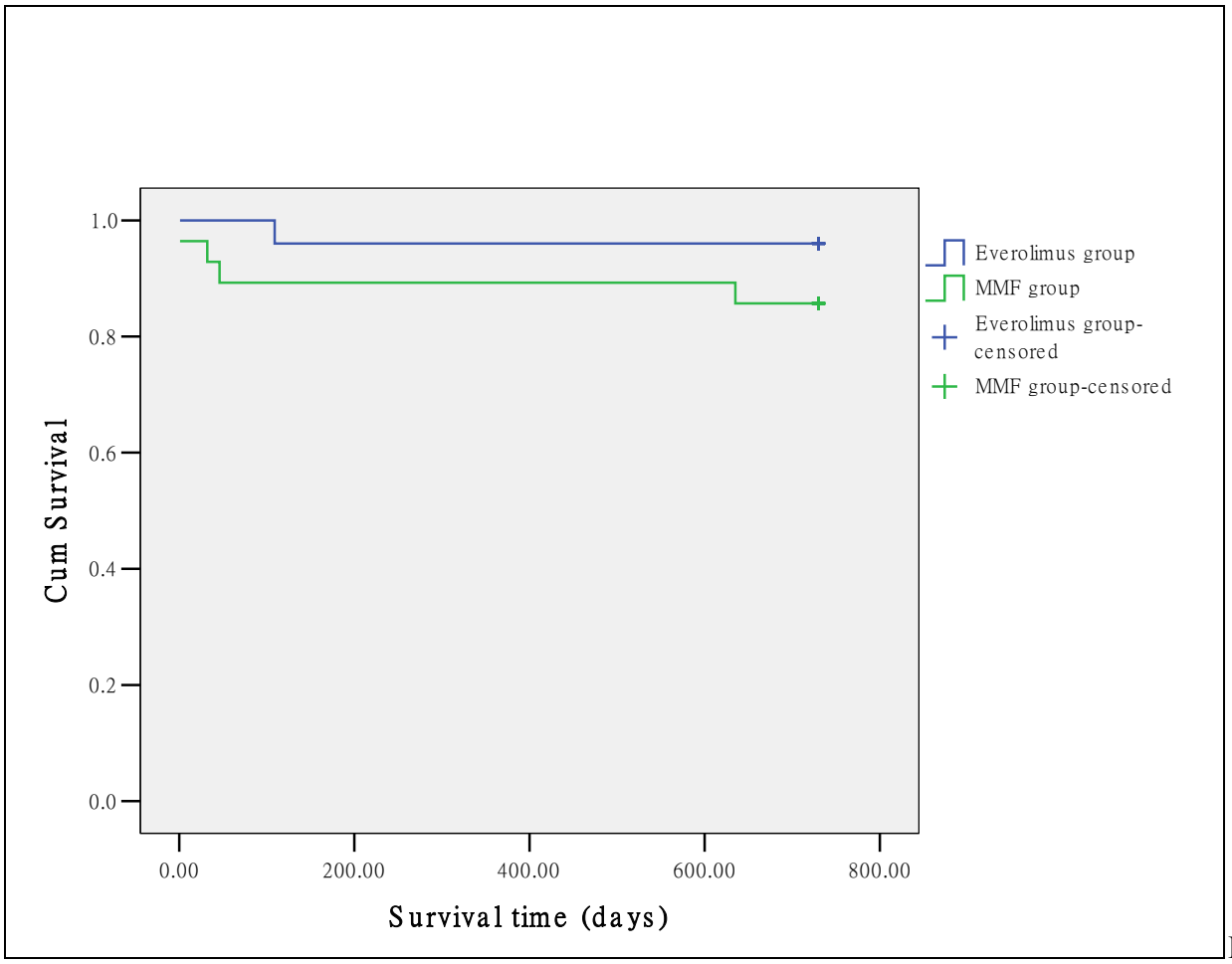

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Figure 3. Survival effect

4.3.2 Effect on acute rejection

Clinically suspected rejection was monitored by cytoimmunology with specific T-cell subcounts and transthoracic echocardiography (including pulsed-wave tissue Doppler evaluation) was conducted when rejection was suspected on the basis of daily noninvasive monitoring of the IMEG.

Nineteen patients in each group experienced rejection (p=0.359) that was detected by IMEG in the first 2 years after transplantation. Each of the first clinical rejection episodes after IMEG implantation was detected by IMEG.

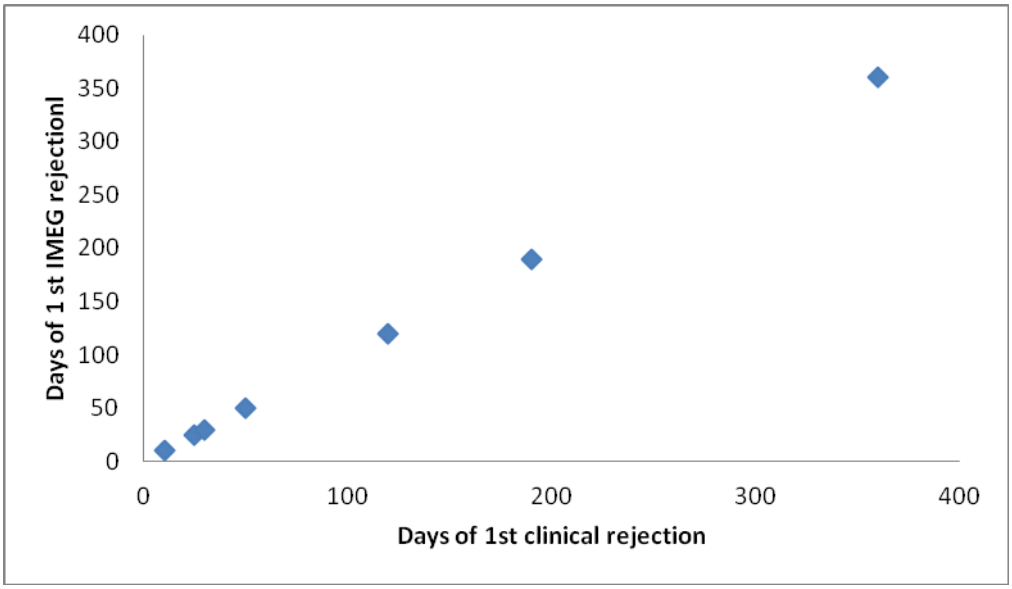

Figure 4. The relationship of IMEG detected rejection ans clinical rejection

Clinically suspected rejections occurred 12 patients in the everolimus group and 22 patients in the MMF group (p=0.043) during the first 2 years after transplantation; most of the rejections occurred in the first 3 months post-transplant.

Eight of the 24 patients in the everolimus group had at least one episode of clinically suspected rejection by the first month post-transplant, and there were 9 cases of least one episode of clinically suspected acute rejection in the first 3 months post-transplant.

There were 15 patients with at least one episode of clinically suspected acute rejection in the first month post-transplant, and 17 of 28 patients had at least 1 episode of clinically suspected rejection by 3 months post-transplant in the MMF group.

Table 9. Clinical rejection, IMEG rejection and CAV

Time post-op	MMF group	Everolimus group	p
In 1 months	15 patients	8 patients	0.143
In 3 months	17 patients	9 patients	0.095
In 1year	19 patients	12 patients	0.029
In 2 years	22 patients	12 patients	0.043
BPAR≥3A	2 cases	2 cases	1.000
IMEG	19 cases	19 cases	0.359
CAV	5(10m,15m,17m,23m,24m)	4(6m,7m,12m,24m)	1.000

During the 2 year follow-up, most cases of biopsy proven acute rejection (BPAR) were mild or moderate in severity. Biopsy indicated 5 cases of ISHLT Grade 1A, and 3 cases of ISHLT Grade 1B in the everolimus group. ISHLT Grade 1A rejection 6 cases, and ISHLT Grade 2 in 1 case was found in the MMF group. There were two patients who experienced ISHLT Grade 3A rejection in each group (p=1.000).None of the acute rejections were associated with hemodynamic compromise and they were easily managed with steroid pulse therapy, ATG or OKT3.

4.3.3 Effect on CAV

CAV was detected by coronary angiography.

Although IVUS is more sensitive in detecting the presence of atherosclerosis in heart transplant recipients, it was not used in children but only in adults who underwent heart transplantation in our center.

CAV was found in 4 cases by angiography in the everolimus group while 5 cases occurred in the MMF group during the 2 year follow-up (p=1.000). The incidence of CAV was comparable between the two groups. However, these data were based on evaluation of angiographic studies from 13 patients.

4.4 Safety

4.4.1 Effect on renal function

There were no significant statistical differences between the groups except that the mean creatinine levels in the everolimus group were significantly higher pre-transplant (p=0.044) and by the first week post-transplant (p=0.003).

Mean creatinine values pre-transplantation were 0.96±0.76 mg/dl, then slightly increased during the first and second months in the everolimus group (the highest value at week 3 post-transplant, 1.42±1.84mg/dl), but the creatinine values decreased after the third month and remained stable in the range of 0.80-1.10±0.23 mg/dl in the 2 years after heart transplantation.

There was also a slight increase in creatinine levels in the MMF group from 0.63±0.32 mg/dl prior to heart transplantation to the highest value of 0.99±0.27 mg/dl at month 24 post-tranplantation.

Table 10. Creatinine values (mg/dl)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	22	23	22	17	12	16	12	10	10	8	9	10	5	6	2
	Mean	0.96	1.19	1.07	1.42	1.09	1.05	0.86	0.89	0.87	0.81	0.81	0.84	0.80	0.97	1.10
	SD	0.76	1.02	1.27	1.84	1.38	1.27	0.45	0.28	0.36	0.30	0.35	0.40	0.32	0.37	0.23
MMF Group	*n*	27	24	24	23	20	12	5	6	6	5	7	14	7	13	2
	Mean	0.63	0.46	0.53	0.48	0.48	0.71	0.96	0.79	0.76	0.83	0.61	0.77	0.87	0.86	0.99
	SD	0.32	0.30	0.32	0.21	0.22	0.44	0.35	0.31	0.36	0.31	0.26	0.37	0.29	0.33	0.27
	t	20.69	3.262	1.997	2.082	1.513	0.886	0.462	0.667	0.574	0.110	1.242	0.453	0.429	0.648	0.443
	P	0.044	0.003	0.052	0.053	0.158	0.384	0.651	0.516	0.575	0.914	0.235	0.655	0.677	0.525	0.701

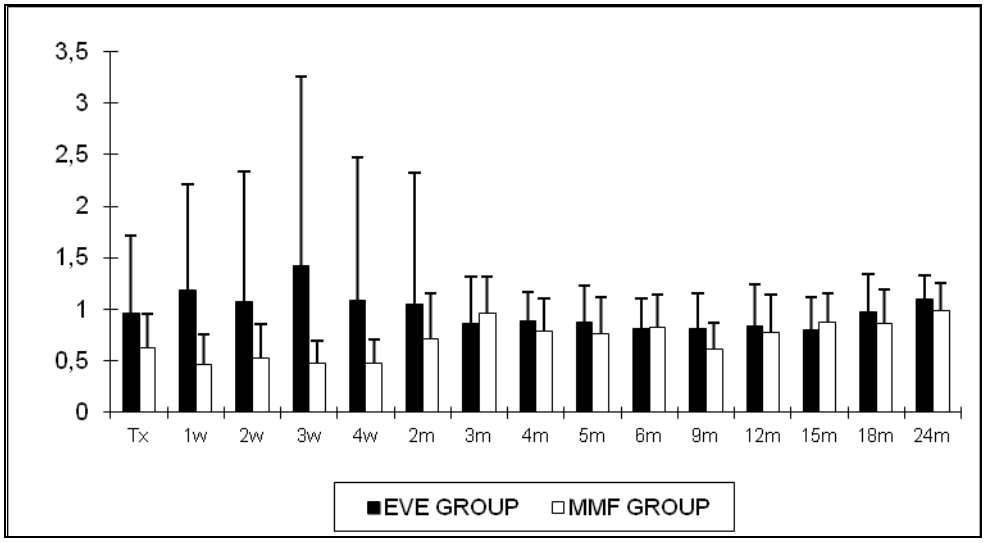

Figure 5. Creatinine values (mg/dl)

Glomerular filtration rate (GFR) was statistically significantly higher in the MMF group at transplant (p=0.048), 1 week (P=0.002) and 3 weeks(p=0.004) following transplantation, but there were no significant differences between the two groups thereafter until 24 months after heart transplantation.

GFR was estimated using the Schwartz formula[70]: eGFR(ml/min/1.73m^2)=kl/serum creatinine, where k is a constant of proportionality, l is body length(cm), and serum creatinine is expressed in milligrams per deciliter (mg/dl).

The values of GFR increased in both groups directly after transplantation, then there was a slight decrease in GFR early after transplantation but it remained stable after months 3 or 4, and throughout the 2 years post-transplant.

Table 11. Glomerular filtration rate (ml/min/1.73m^2)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	22	23	22	17	12	16	12	10	10	8	9	10	5	6	2
	Mean	103.6	112.4	132.9	106.3	127.7	120.1	114.1	111.9	95.3	103.0	91.1	102.0	108.4	90.5	95.1
	SD	2.6	85.2	79.7	59.5	70.7	69.9	58.5	65.3	28.2	49.0	32.5	26.5	22.2	23.5	0.3
MMF Group	*n*	27	24	24	23	20	12	5	6	6	5	7	14	7	13	2
	Mean	140.6	188.1	180.5	176.0	160.2	141.3	86.9	92.1	101.8	83.5	106.4	100.9	101.6	95.1	88.9
	SD	81.6	71.5	92.9	80.1	71.2	90.0	16.2	28.4	40.1	11.8	45.3	43.2	27.3	29.9	5.2
	t	2.043	3.302	1.861	3.21	1.252	0.702	1.482	0.697	0.383	0.860	0.788	0.071	0.456	0.331	1.711
	P	0.048	0.002	0.069	0.004	0.220	0.489	0.160	0.497	0.708	0.408	0.444	0.944	0.658	0.745	0.229

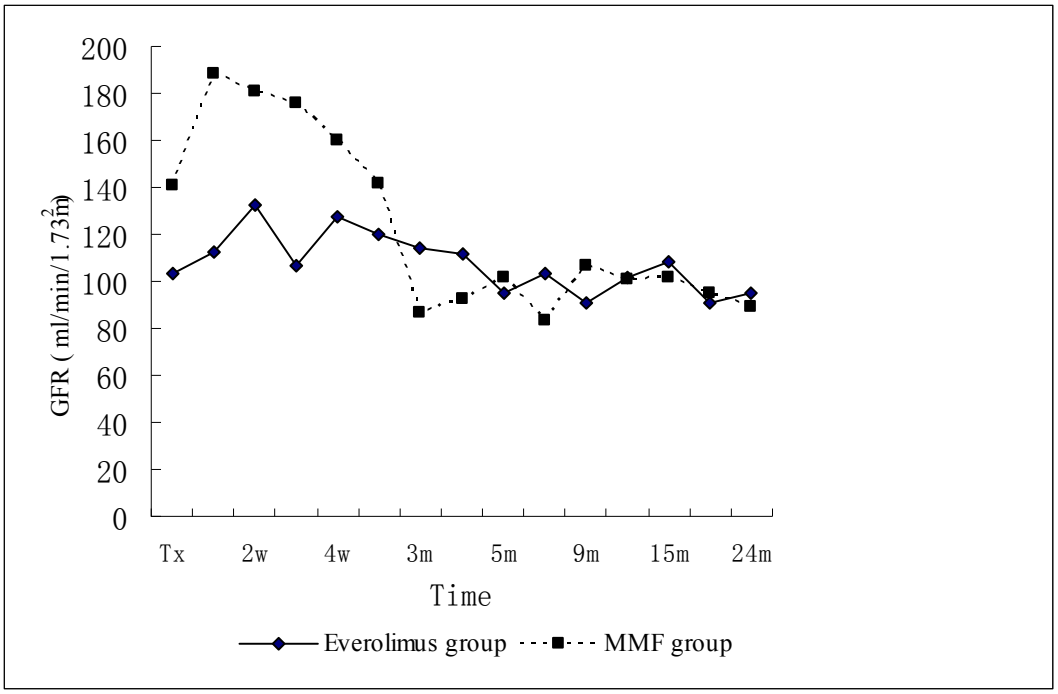

Figure 6. Glomerular filtration rate (ml/min/1.73m^2) over time

4.4.2 Effect on hematology

The mean white blood cell count in both groups increased to more than 10000/ml in the first 2 to 3 weeks after transplantation, then decreased to the normal range; no leucopenia was observed throughout the 24 months follow up.

Table 12. White blood cell (x10^9 /L)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	24	23	24	19	14	17	12	12	7	6	9	11	5	6	3
	Mean	11.8	11.7	13.2	10.2	8.7	8.0	8.7	7.6	7.8	5.8	7.8	7.8	7.8	9.0	8.8
	SD	5.7	4.6	5.9	5.3	4.6	3.5	4.0	2.8	3.3	1.9	3.5	3.6	3.4	3.6	4.0
MMF Group	*n*	26	24	25	23	19	15	8	7	6	4	6	15	7	12	2
	Mean	13.1	12.2	13.8	9.2	12.4	8.0	7.6	5.7	6.7	6.1	6.3	7.0	6.7	7.3	7.3
	SD	7.8	4.8	5.4	4.7	11.7	4.2	3.2	1.9	3.7	1.4	2.6	3.1	2.3	3.2	0.02

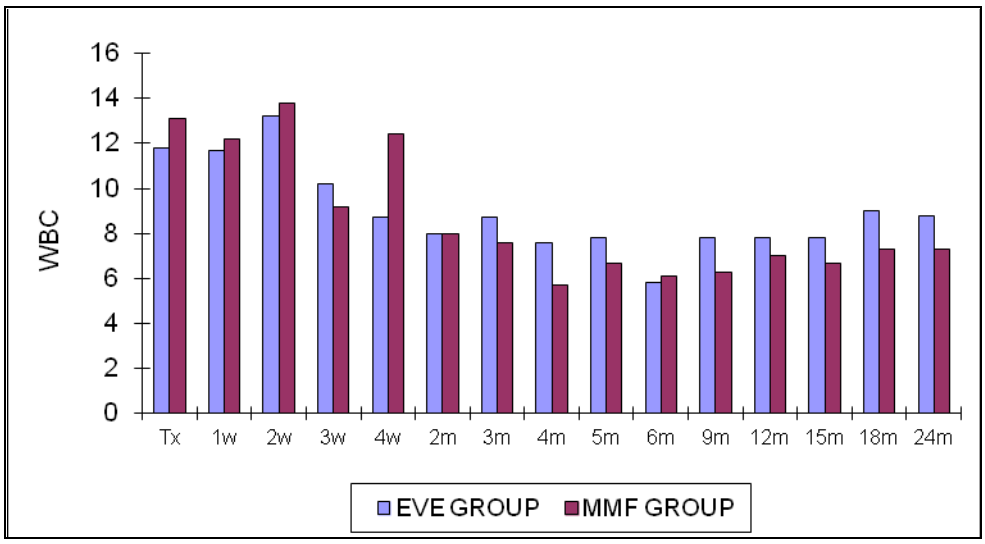

Figure 7. White blodd cell (x10^9 /L)

Hemoglobin concentration was similar in both groups at baseline and at 6, 12 and 24 months after heart transplant. The difference between the two groups was not statistically significant. Hemoglobin concentrations were significantly lower at week 3 post-transplant, probably because the patients were stable, and more fluid intake diluted the blood but no transfusion nor

erythropoetin administration was necessary. The values of hemoglobin increased to the lower normal range thereafter.

No severe anemia (<8g/L) was noted during the following 2 years.

Table 13. Haemoglobin (g/L)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	25	25	25	23	18	22	16	19	11	16	16	15	10	12	2
	Mean	11.87	11.66	10.84	10.18	11.01	11.30	11.03	11.37	11.15	11.36	11.49	11.61	11.67	11.23	10.85
	SD	1.61	1.85	1.72	1.26	1.25	1.38	1.79	1.41	1.29	1.32	1.46	1.29	1.28	1.53	0.35
MMF Group	*n*	27	26	26	24	23	17	11	9	9	9	9	16	13	16	13
	Mean	12.61	11.37	10.66	10.21	11.06	11.46	11.43	11.20	10.80	11.74	10.49	11.42	11.81	11.16	11.34
	SD	3.62	1.77	2.01	1.67	1.60	1.35	0.96	0.77	1.48	1.13	1.48	1.36	1.55	1.48	1.76

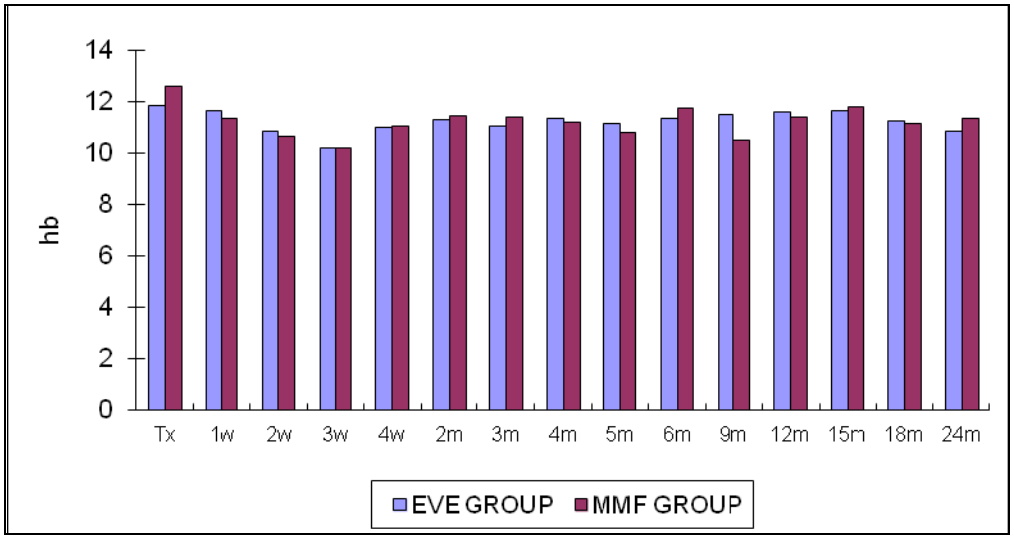

Figure 8. Haemoglobin (g/L)

The platelet count was not impacted as adverse effects and the values were maintained within the normal range (no thrombocytopenia) in the 2 years post-transplant. The differences between the groups were not significant throughout the 24 months post-transplant except at week 4 (p=0.019).

Table 14. Platelet count (x10^9 /L)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	25	23	25	20	14	17	14	12	8	8	9	10	6	7	3
	Mean	168.8	228.4	377.1	385.1	283.0	305.9	311.5	292.4	321.4	280.5	340.0	306.1	316.0	331.3	303.0
	SD	80.9	158.2	246.0	186.1	103.7	167.3	176.3	69.0	143.1	120.4	196.1	141.2	72.8	75.5	78.1
MMF Group	*n*	25	24	24	23	18	13	9	7	7	6	5	14	9	12	2
	Mean	227.7	269.9	448.7	376.9	398.0	371.1	349.2	316.7	386.9	419.1	409.4	289.9	285.2	369.3	294.0
	SD	120.5	135.9	199.2	109.4	148.0	109.4	102.4	41.0	178.2	312.0	136.2	73.2	72.5	186.2	149.9

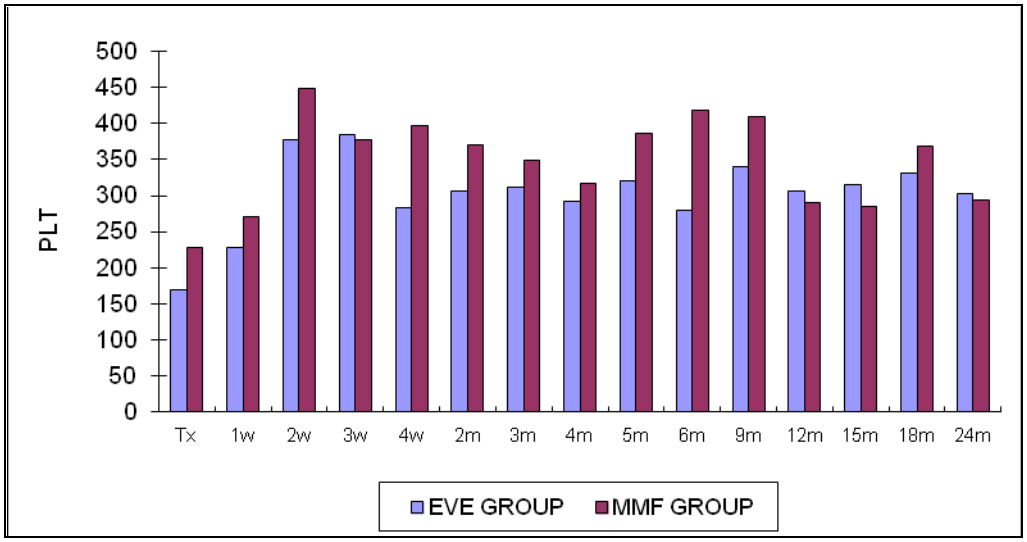

Figure 9. Platelet count (x10^9 /L)

4.4.3 Effect on lipids

No significant differences were noted between the two groups. All the recipients on everolimus received statin therapy (fluvastatin or pravastatin 5-40 mg/day) soon after transplantation, and the total cholesterol levels were generally well controlled at relatively high but acceptable values (normal value was defined as less than 175 mg/dl). Hyperlipidemia was observed in the everolimus group in the first 4 months after heart transplantation; the mean total cholesterol level increased from 169 at week 1 to 274mg/dl at week 4, but no severe hyperlipidemia occurred. The mean serum cholesterol levels were well controlled in the normal range after the 5th month until 24 months after transplant. No pronounced hyperlipidemia was noted in the MMF group.

Table 14. Cholesterol (mg/dl)

		Tx	1w	2w	3w	4w	2m	3m	4m	5m	6m	9m	12m	15m	18m	24m
Everolimus Group	*n*	0	1	2	3	2	13	9	7	5	6	9	8	4	6	3
	Mean		169	235	224	274	249	222	234	201	199	206	193	200	189	177
	SD			131	45	65	67	64	35	52	51	64	47	59	39	6
MMF Group	*n*	0	2	1	1	1	8	1	5	5	2	4	10	6	9	2
	Mean		169	222	173	197	204	249	194	164	244	178	191	180	179	151
	SD		0						64	30	14	40	40	39	30	38

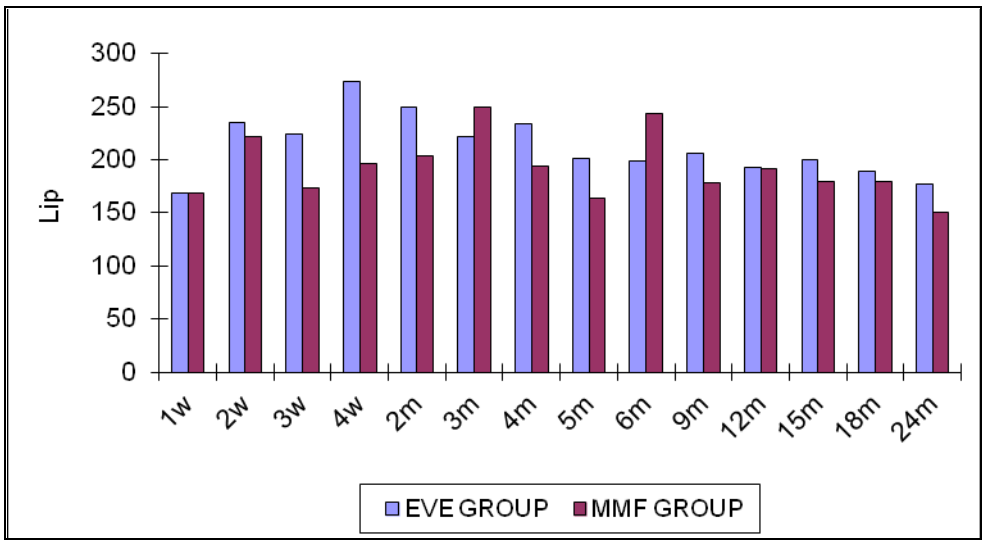

Figure 10. Cholesterol (mg/dl)

4.4.4 Effect on infection and lymphoproliferative disorders

One case in the everolimus group and four cases in the MMF group had CMV infection(p=0.358).

Wound infections or delayed wound closure were encountered in one case in the everolimus group and 5 cases in the MMF group (p=0.199), although everolimus has anti-proliferative properties.

One patient in each group developed lymphoproliferative disease (p=1.000).

4.5 ECG, echocardiography and CAG

The ECGechocardiography and CAG data were comparable between the two groups.

The ventricular function data obtained by echocardiography were well preserved and remained stable in all survivors throughout the 2 years post-transplant except when acute rejection happened.

5 Discussion

To the best of our knowledge, this is the first retrospective study comparing the safety and efficacy of everolimus versus MMF in combination with cyclosporine and corticosteroids in paediatric cardiac transplantation.

The main conclusions from our study are that both regimens are efficacious and appear to be well tolerated in paediatric heart transplant recipients. An everolimus based immunosuppresive regimen offers effective immunosuppression with reduced BPAR and CAV occurrence with comparable patient survival, renal function is preserved with reduced cyclosporine exposure facilitated by everolimus, the haematological abnormality is mild and hyperlipidemia occurred but is well managed during the 2 years after heart transplantation.

Innovations in immunosuppression therapy in heart transplantation often originate from renal transplantation, given the larger volume and greater experience. Everolimus was also first clinically introduced into renal transplantation [71], and later reported in heart transplant recipients [65].

Pharmacokinetics and dosage of everolismus

Therapeutic drug monitoring would be a helpful means for individualizing everolimus exposure to reach the balance between over- and under-immunosuppression, assessing regimen adherence, and adjusting doses as the child matures. Increasing evidence from kidney transplantation also suggests that everolimus therapy should be guided by therapeutic drug monitoring[14].

A better understanding of pharmacokinetic parameters would help reduce drug exposure through more accurate drug monitoring and management of CNI dose to minimize side-effects and improve outcomes.

Paediatric patients often have altered pharmacokinetic profiles of immunosuppressive drugs, reflecting limited absorption, altered metabolism, or a more rapid drug clearance, which necessitate dosage adjustments targeted for optimal outcomes [72].

Also, there is pharmacokinetic data indicating that paediatric patients require a dose adjusted to body size (either mg/kg or mg/qm) to achieve similar exposure as adults[73].

The results from our observations support the use of a 0.8-1.2mg/qm dosage of everolimus in paediatric heart transplant recipients, which is in accordance with the data reported in paediatric renal transplant patients[74].

The mean daily dose of everolimus was 1.16±0.56 mg/qm body surface area. Everolimus trough levels were 5.08±2.42 ng/ml 1 week after administration of 0.8-1.2 mg/qm of everolimus in paediatric heart transplant recipients, and very stable trough blood levels were maintained at the middle of the optimal therapeutic window of 3-8 ng/ml throughout the 24 month following up.

No difference regarding pharmacokinetics was noted in groups with different age (age stratification separating infants from older children), body weight, body surface area or gender.

Everolimus was initially investigated for its anti-proliferative property; the immunosuppressive effect was disclosed during further evaluation.

Everolimus inhibits growth-factor-dependent proliferation of cells through a calcium-independent signal [56], whereas CsA inhibits T-cell-dependent growth factors through a calcium-dependent signal. Everolimus combined with cyclosporine and methylprednisolone significantly reduces T-cell proliferation [57].

Everolimus exerts its effect at a different physiological target to CsA, raising interest in an immunosuppressive strategy that combines both drugs that act synergistically via complimentary mechanisms of action to optimize exposure of both agents such that the side-effects of either everolimus or CsA are reduced while achieving high efficacy.

Synergistic activities between everolimus and cyclosporine have been reported in animal models of organ transplantation. Combination therapy using 10% of full everolimus dose with 30% of full CsA dose was as effective as administering either drug alone at full dose [49].

The complementary mechanisms of action of everolimus and CsA, resulting in a synergistic immunosuppressive effect that allows for the reduction of CsA trough blood levels in heart transplant recipients, an approach that potentially has the dual benefit of reducing risk of nephrotoxicity and CAV without any added risk of acute rejection [6], is consistent with the experience from several renal studies [58,75]. Therapeutic drug monitoring studies in both renal and heart transplantation have shown that everolimus trough blood levels of at least 3 ng/mL are

required to ensure optimal therapeutic effects [51]. Everolimus trough blood levels of 3 to 8 ng/mL were associated with a 2.5-fold lower rate of rejection than levels < 3 ng/mL ($p < 0.0001$), while trough blood levels > 8ng/mL did not confer any extra efficacy benefit [50]. Thus, before reducing CsA trough blood levels, everolimus trough levels of 3 to 8 ng/mL should be achieved. Normally, stable trough blood levels within the desired range could be achieved within 1 to 2 weeks of initiating everolimus. In a single center experience, using concentration-controlled everolimus to minimize CsA exposure is positive [65]. A 25% to 30% reduction in CsA trough blood levels for patients receiving everolimus was thought reasonable without compromising immunosuppressive efficacy [41]. Everolimus and basiliximab therapy in renal transplant recipients meant that CsA exposure could be reduced by approximately 50% in the months following transplantation [53]. The combination of these two approaches could be an appropriate course of treatment for heart transplant patients with preoperative renal dysfunction [41]. A study by Rothenburger et al. showed the initial clinical results of CNIs-free immunosuppression to be promising and may provide improved immunosuppressive therapy in the future [76]. The 2 year survival rates were 86% in the MMF group and 96% in the everolimus group ($p=0.200$). Eight patients in the MMF group and 10 patients in the everolimus group were on either univentricular or biventricular assist device support as a bridge to heart transplantation. With the life-saving mechanical assist devices, these critically ill children with intractable end-stage heart failure who often would die within hours received the

chance to be bridged to transplantation after weeks and months until the delivery of an appropriate donor heart.

The introduction of CNIs in the early 1980s has been considered the cornerstone of immunosuppressive medication for many years, and they are likely to remain the foundation of immunosuppressive therapy for the foreseeable future.

Recently introduced potent immunosuppressive agents have enhanced short-term results after transplantation but failed to influence allograft vasculopathy and long-term graft and patient survival [77]. Therefore, there is a pressing need for immunosuppressive strategies that prevent both acute rejection and vascular proliferation leading to late graft loss.

Acute rejection remains a significant problem in paediatric heart transplant populations. The risk of rejection was surprisingly low in the 2 years post-transplant in both groups. The immunosuppressive safety profile was well maintained as everolimus in combination with cyclosporine and corticosteroids was associated with a lower surveillance biopsy proven acute rejection cumulative rate of 50% at year 1 and 50% at 2 years, compared with 67.9% and 78.7% at years 1 and 2 in the MMF group, respectively (p=0.029, p=0.043). But BPAR ≥3A occurred in two cases in each group (p=1.00).

Acute rejection and cardiac allograft vasculopathy account for most of the late deaths after heart transplantation. Plenty of evidence demonstrated the importance of early detection and treatment of acute rejection episodes. Day by day monitoring of the heart with IMEG can recognize acute rejection at an early stage and improve the allograft function and long-term survival.

Noninvasive measures such as IMEG, conventional echocardiography and tissue Doppler echocardiography used at the Deutsches Herzzentrum Berlin for rejection detection were reliable. Indicative biopsy was performed other than protocol biopsy. IMEG was highly sensitive in detecting acute rejection at an early stage, so prompt treatment prevented the development of rejection to more severe stage.

1. The IMEG devices were implanted mostly at the time of transplantation. Some patients who were on VAD support had implantation of the IMEG system once the wound had healed at 2 to 304 days post-transplant to reduce the risk of infection. Many of the clinical rejection episodes happened before the IMEG started to work.

2. After the IMEG started to work, none of the clinical rejection episodes was missed by the IMEG devices - this device is highly sensitive.

3. The specificity of the IMEG is moderately highly sensitive. There are some rejection episodes indicated by IMEG (most happened several hundreds days post-transplant)that were not diagnosed as clinical rejection. That means that some false-positive results of the IMEG voltage drop happened late post-transplant, maybe due to pericardial fluid, infection or scar.

Combined with echocardiography, the non-invasive protocol was superior to biopsy, which had inherent deficiencies, such as sampling error and overlooking humoral rejection. The risk of

early acute rejection is gradually receding because of the significant advances in the new immunosuppressive regimens. However, CAV continues to be the major risk factor for mortality after the first year of cardiac transplantation. CAV has a complex, multi-factorial etiology. The novel anti-proliferative immunosuppressive agent everolimus may tackle it because smooth muscle cell proliferation is central to the pathogenesis of CAV.

Everolimus can decrease vascular remodelling by preventing growth-factor-mediated smooth muscle cell proliferation and attenuating vascular neointimal formation in animal models [78]. The potential for reducing intimal hyperplasia in coronary arteries in animal models of immune and nonimmune injury is also being realized in humans [45].

Chronic allograft dysfunction is the major barrier to long-term graft and patient survival after transplantation. This is a multifactorial process in which the primary causes are acute rejection, vascular remodeling, CNI induced nephrotoxicity and CMV infection [79,60]. Everolimus prevents vascular remodelling and neointimal proliferation, which are both key components of CAVas well as suppressing T cells.

Everolimus has been shown to be associated with reduced incidence of acute rejection and lower rates of CMV infection [64,46,40], both of which are risk factors for the development of CAV in heart transplantation [47,48].

Also, everolimus has demonstrated efficacy for preventing CAV and its progression [64,46].

Because it can target several of these contributory factors, everolimus has the potential to improve long-term outcomes in transplantation[80].

One important factor to decide on the long-term or de novo use of the everolimus regimen lies in its impact on prevention of cardiac allograft vasculopathy by suppressing the two main causes of CAV, rejection and smooth muscle cell proliferation, given the fact that CAV is a principal cause of morbidity and mortality after the first post-transplant year[81]. The efficacy of everolimus in preventing vasculopathy in heart transplant recipients is beneficial to those already with CAV or who exhibit high risk factors for coronary artery disease.

The beneficial effects of everolimus on CAV appear to be PSI class specific, since studies have demonstrated that sirolimus can also slow CAV progression[82].

IVUS is a sensitive technique for the early detection of vasculopathy[83].

Progression of intimal thickening of ≥0.5 mm in the first year after transplant is a reliable surrogate marker for subsequent development of CAV and mortality after heart transplantation[27].

However, IVUS was not used in paediatric recipients in our cohorts.

Everolimus, in combination with CsA, is efficacious for reducing the incidence of acute rejection and cardiac allograft vasculopathy in cardiac transplantation recipients and survival is improved significantly. Prolonging survival allows more time for the adverse effects to manifest; the CsA associated renal dysfunction prevents the administration of

sufficient doses of the drug to fully exploit the immunosuppressive potential in transplantation.

To permit CNIs dose reduction and thereby mitigate renal dysfunction, one approach sought to discover an agent that can act in synergy with CNIs. Everolimus appears to have no nephrotoxic activity, but it enhances CNI induced nephrotoxicity, reported as increased serum creatinine levels, especially when combined with high-exposure CsA[73]. However, everolimus and CsA have complementary mechanisms of action, resulting in a synergistic immunosuppressive effect that may enable the dose of CsA to be reduced without loss of efficacy [49]. Cyclosporine diminishes the rate of acute rejection, but nephrotoxicity of cyclosporine has also been well documented in both heart and kidney transplant patients[84].

Because the nephropathy caused by CNIs can be acute or chronic, dose- related, a lower dose of CNIs would potentially decrease the renal damage produced by these drugs and result in improved renal function[85]. It is important to monitor the renal function in heart transplant patients receiving CNIs. Serum creatinine level of 2 mg/ml should be the indication for conversion to everolimus. Antibody induction therapy with everolimus at transplantation, followed by CsA initiated 5-7 days later at a lower CsA exposure by a reduction of up to 50% may provide effective renal protection for patients with preoperative renal dysfunction[41]. The first results of improvement in renal function owing to reduced CNI doses combined with everolimus in heart transplant recipients were reported by Lehmkuhl and Hetzer[64], Lehmkuhl et al.[41], and Zuckerman[40]. Monitoring of serum creatinine levels and calculated GFR is routinely

performed in patients receiving CsA after heart transplantation at the Deutsches Herzzentrum Berlin. However, there are serious limitations to using serum creatinine values to define kidney function in children, because it is known that serum creatinine varies significantly with age. Also, changes in serum creatinine levels and GFR are poorly correlated and a small change in serum creatinine levels may translate into a large change in GFR.

Therefore, assessment of both serum creatinine levels and GFR is advised, although measured GFR is currently considered the most reliable parameter of kidney function.

Everolimus alone has not shown any renal toxic effect in pre-clinical studies or in humans[86,87]. Everolimus, in combination with reduced levels of cyclosporine, allows for minimized nephrotoxicity without increasing the risk of immunological events, which impacted the allograft survival.

Patients with established or anticipated renal dysfunction may benefit more from the lower CsA exposure facilitated by the everolimus regimen.

Although serum creatinine levels increased slightly early post-transplant, a direct correlation between creatinine levels and CsA exposure was noted in our study, with progressive reductions in CsA exposure being accompanied by decreases in creatinine levels.

GFR increased soon after transplantation, which may be related to improved ventricle function and renal perfusion after heart transplant, followed by a slight decline in GFR that may be explained by the nephrotoxicity of CsA, but values stabilized from month 3 or 4, and throughout the 2 years after heart transplantation. Studies in adult cardiac transplant patients also reported an early decline in renal function, followed by a plateau[88,89]. The renal function was well preserved in the everolimus group as with the MMF treated patients in our study. We also found a correlation between CsA exposure and GFR. A study in paediatric heart transplant by Hornung et al reported that the decline in renal function correlated with early cyclosporine exposure and persisted even when doses were subsequently reduced[90]. Another study by Rice et al reported that a late cyclosporine reduction did not improve renal function after the first year post-transplant[91]. The nephrotoxicity of cyclosporine enhanced by everolimus was successfully avoid by reduced dosage of cyclosporine.

Our findings support the following studies. The clinical experience following kidney transplantation has shown that everolimus facilitates reduced exposure to CsA, and that the everolimus plus low-exposure CsA regimen preserves renal function without compromising immunosuppression efficacy[53,52]. Early clinical experience with everolimus in heart transplantation has indicated that the agent can also be used with low-exposure CsA in heart transplant recipients, maintaining renal function without loss of immunosuppressive efficacy[64,92].

The side-effect on haematology was mild in both everolimus and MMF treated patients. Haematologic abnormalities, such as anaemia, leukopenia, and thrombocytopenia were not observed in our study.

Blood lipids should be closely monitored in patients receiving everolimus, because impairment of lipids was observed in most patients receiving the drug[85]. It is known that CNI also induces marked hyperlipidemia[93]. In the case of hyperlipdemia, a well chosen statin should be prescribed. In consistency with the known side-effects of mTor-inhibitor, hypercholesterolemia was noted soon after everolimus administration, but serum cholesterol levels were manageable over the 2 years post-transplant by the routine statin treatment. Lipid lowering agents can not only inhibit cholesterol production, but also reduce the risk for developing cardiac allograft vasculopathy, because transplant vasculopathy is one of the major problems limiting long-term survival. Generally, immunosuppressive therapy is associated with an increased incidence of malignancy and infections, mostly opportunistic infections.

The incidence of CMV infection was very low in everolimus treated patients (4% in the everolimus group, 14% in the MMF group, p=0.358), which is consistent with the results of the study by Eisen et al. in the Phase III trial[94]. Although the data in our study did not reach a significant difference, a possible explanation may be the relatively small number of patients. A similar result was seen in the study of kidney-allograft recipients[95]. Because CMV infection may increase the morbidity and mortality in transplant recipients a lower incidence of CMV infection has special advantage for the patients with CMV mismatch (donor positive/recipient negative); also re-transplant patients are good candidates for everolimus.

Although cyclosporine has greatly improved survival after organ transplantation, acute and chronic rejection as well as adverse effects continue to be of concern, particularly in children who potentially face many decades of therapy. Adverse effects include renal dysfunction, hypertension, hyperlipidemia, hirsutism, gingival hyperplasia and abnormal facial bone growth [96].

CNIs are associated with arterial hypertension[89]. Lower doses of CNIs may help bring the elevated blood pressure under control. No severe arterial hypertension was encountered in our study, maybe due to the effective anti-hypertension therapy.

Despite the improvements in immunosuppression therapy, the most advantageous combination of immunosuppressive agents associated with the best outcome for survival, rejection and safety in cardiac transplant recipients has not yet been established.

Because this study was based on retrospective and nonrandomized analysis of data, the small number of patients limited our analysis and precludes any definitive statement regarding which drug regime may be more efficacious; the results are therefore of a preliminary nature . The efficacy and safety profile of everolimus has to be evaluated in ongoing prospective long-term randomized larger samples trials.

Nevertheless, this study suggests that the use of 0.8-1.2mg/qm everolimus in paediatric heart recipients may maintain everolimus trough levels around the middle of the optimal therapeutic window of 3-8 ng/ml, which allows the trough concentration of cyclosporine to be markedly reduced by 23% to 53%. The efficacy profile regarding acute rejection and CAV was comparable with that of MMF and renal function was well preserved. Effects on haematology were mild; hyperlipidemia was observed in everolimus treated patients but could be well managed.

6 Conclusion

Everolimus 0.8-1.2 mg/qm, adjusted to maintain trough blood levels of 3-8 ng/ml, is the recommended dosage in paediatric heart recipients.

Everolimus, as part of the primary immnunosuppressive regimen, allows CsA exposure to be markedly reduced in paediatric heart transplant recipients, preserving renal function with the main effect (prevention of acute rejection) of any immunosuppressive agent being achieved. Everolimus also demonstrated its antiproliferative properties in preventing the occurrence of cardiac allograft vasculopathy. The safety profile of everolimus is also demonstrated in our study. Because of the promising mid-term results in de novo heart transplant patients, everolimus in combination with a reduced dose CsA and steroid regimen is also conducted in maintenance heart transplant recipients intolerant to the current regimen or in whom the regimen proved insufficient. Some transplant physicians are considering everolimus an effective means of reducing or avoiding CNI.

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Eidesstattliche Erklärung

“Ich, Haibo Wu, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: “Immunosuppression with Everolimus in Paediatric Heart Transplantation” selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe „Uniform Requirements for Manuscripts (URM)“ des ICMJE-www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o.) und werden von mir verantwortet.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o.) und werden von mir verantwortet.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

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List of publications

Wang H, Wu H, Jian H, Wang Z, Potapov E, Stepanenko A.

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