

Diploma Thesis

**The Influence of mTOR Inhibitors on CMV
Recurrence after Whole Organ Transplantation**

submitted by

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(signature)

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Zusammenfassung

EINFÜHRUNG: Das Cytomegalievirus (CMV), ein Mitglied der β - Herpes-Viren Gruppe, stellt eine der wichtigsten Infektionen nach Organtransplantationen dar. Im Gegensatz zu Immunkompetenten verläuft eine CMV-Infektion bei Immunsupprimierten dramatisch und beeinflusst deren Morbidität und Mortalität. mTOR Inhibitoren, vertreten durch Sirolimus und dessen Derivat Everolimus, sind eine neue Substanzklasse immunsuppressiver Medikamente, die in der Transplantationsmedizin verwendet werden. Unser Hauptziel war es, herauszufinden ob es Unterschiede zwischen Sirolimus und Everolimus in Bezug auf das Auftreten von CMV Infektionen gibt.

METHODEN: Eingeschlossen wurden Patienten, die zwischen 1983 und Dezember 2008 an der klinischen Abteilung für Transplantationschirurgie der Universitätsklinik für Chirurgie im LKH Graz, Herz (HTX) oder Leber (LTX) transplantiert wurden. Eine Therapie mit Sirolimus oder Everolimus stellte das Einschlusskriterium dar. Es wurden retrospektiv Daten über die CMV Infektionen, den CMV Status des Spenders und Empfängers und die Konzentrationen der Immunsuppression erhoben und statistisch ausgewertet.

ERGEBNISSE: In diesem Zeitraum entwickelten 2 HTX Patienten (5%) und 20 LTX Patienten (29%) CMV Infektionen. 9,1% der CMV infizierten Patienten erhielten Everolimus, 90,9% Sirolimus. Patienten, die Sirolimus erhielten, entwickelten signifikant öfter eine CMV Infektion als solche, die Everolimus bekamen ($p=0,003$). Insgesamt entwickelten signifikant mehr LTX Patienten eine CMV Infektion als HTX Patienten. Der CMV Status des Spenders oder des Empfängers vor Transplantation hatte keinen Einfluss auf das Auftreten einer CMV Infektion.

DISKUSSION: Da es keine Studien gibt, die Sirolimus und Everolimus in Bezug auf das Auftreten einer CMV Infektion vergleichen, konnten wir unsere Resultate nicht mit anderen vergleichen. Die Infektionsraten von Everolimus sind anderen Studien ähnlich, die von Sirolimus nicht. Die Infektionsraten von HTX und LTX Patienten korrelieren nicht mit anderen Studien. Möglicherweise gibt es ein Bias in unserer Studie, aufgrund der Tatsache, dass die meisten LTX Patienten Sirolimus und die meisten HTX Patienten Everolimus erhielten. Ein Vergleich mit anderen Studien ist aufgrund verschiedener immunsuppressiver Schemata und Studiendesigns schwierig. Die Größe unserer Studie reicht nicht aus, um eine definitive Tendenz zu zeigen, deshalb sollten weitere Studien durchgeführt werden.

Abstract

INTRODUCTION: Cytomegalovirus (CMV), a member of the β -herpes-virus group, is one of the most important infections after solid organ transplantation. In immunocompetent persons CMV infection is generally harmless. But in immunosuppressed patients it is responsible for serious disease and can influence morbidity and mortality. mTOR inhibitors are a new group of immunosuppressive drugs used in solid organ transplantation, represented by Sirolimus and its derivate Everolimus. Our main intent was to evaluate if there is a difference in occurrence of CMV infection in recipients treated either with Sirolimus or Everolimus.

METHODS: Patients after orthotopic heart transplantation (HTX) and after orthotopic liver transplantation (LTX) transplanted between 1983 and December 2008 at the Medical University Graz, Austria, Department for Surgery, Division of Transplantation Surgery, were included in this study. Receiving either Everolimus or Sirolimus in their immunosuppressive regimen was the inclusion criteria. Data about CMV infections, CMV donor and recipient status and through levels of immunosuppression was collected retrospectively and analysed statistically.

RESULTS: 2 HTX recipients (5%) and 20 LTX recipients (29%) developed CMV infection during this period. From CMV infected recipients 9,1% obtained Everolimus and 90,9% received Sirolimus. In Sirolimus treated patients CMV infection occurred significantly more often than in Everolimus treated ($p=0,003$). Overall, significantly more LTX patients developed CMV infection than HTX patients did. CMV serostatus of the donor or the recipients prior to transplantation had no influence on the occurrence of CMV infection.

DISCUSSION: Because there are no previous studies comparing Sirolimus and Everolimus related to their effect on the occurrence of CMV infection, we could not compare our results with others. Comparing our infection rates with others, the data for Everolimus treated recipients is similar, data for Sirolimus treated does not correlate. Infection rate in our HTX and LTX recipients is different to other studies. There could also be a bias in our study due to the fact that most of LTX patients received Sirolimus and most of HTX patients Everolimus. Anyway, it is difficult to compare our results with others, because of different immunosuppressive regimens and study designs. Besides the size of our study group is too small to see a definite trend, so further investigations have to be performed.

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Abbreviations

CMV	cytomegalovirus
DNA	deoxyribonucleic acid
EBV	Epstein Barr virus
eIF-4E	eukaryotic initiation factor 4E
ELTR	European Liver Transplant Registry
FKBP	FK binding protein
GGT	gamma-glutamyltransferase
GOT	glutamic-oxaloacetic transaminase
GPT	glutamic-pyruvic transaminase
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HSV	herpes simplex virus
HTLV	human T-lymphotropic virus
HTX	heart transplantation
IV	intravenous
IVC	inferior vena cava
LTX	liver transplantation
LVAD	left ventricular assist device
mRNA	messenger ribonucleic acid
mTOR	mammalian target of rapamycin
NYHA	New York Heart Association
PBC	primary biliary cirrhosis
PCR	polymerase chain reaction
PHAS I	phosphorylatable heat stable protein I
PI3K	phosphatidyl-inositol-3-kinase
PNM	polymorphonuclear leukocytes
PSC	primary sclerosing cholangitis
RNA	ribonucleic acid
TOR	target of rapamycin
VZV	varicella zoster virus

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1 Introduction

1.1 Transplantation

1.1.1 Indications of liver and heart transplantation

1.1.1.1 Liver transplantation

In the moment liver transplantation (LTX) is the only option to treat patients with an end-stage liver disease. Compared to other solid organ transplantations (e. g. kidney or pancreas) there are no reliable options in terms of a substitution method for the liver (1).

Because of the rapid progress of new techniques in surgery, improvement in preoperative treatment and immunosuppression, in the last years, the indications for LTX have enlarged, including diseases for which this therapy was not approved before (2). Therefore also the survival rate has improved gradually to a 1-year survival of 83% and a 5 year survival of 75% (3).

According to the ELTR (European Liver Transplant Registry) report, cirrhosis is the most numerous indication for LTX (50%). Its main causes are viral infection (24%), especially hepatitis C infection which represents 16%, and alcohol (18%) (4).

Liver failure due to cirrhosis is evaluated by the Child-Turcotte-Pugh classification. This score is composed by five criteria including serum bilirubin, serum albumin, prothrombin time, ascites and encephalopathy. An increased Child's score is associated with decreased patients survival. This classification is a useful method to estimate the significance of cirrhosis of the liver, because of its simplicity, low costs, availability and its good ability to discriminate (5).

The long-term survival of patients with alcoholic caused cirrhosis is similar to those with non alcoholic causes, but the major problem is, if the patient continues drinking after transplantation or not. And so most centres prescribe abstinence of alcohol for at least 6 months prior to transplantation and strict abstinence after transplantation (6). Though, it could be shown, that only about 5 % of the patients who restart drinking after transplantation, harm their transplants (7).

Up to 20% of the patients infected by the hepatitis C virus (HCV) develop a cirrhosis after 20 years of infection (8).

LTX is not the healing of hepatitis C. In general the virus does return and often damages the new organ. So, controlling and accurate treatment of the virus before and after transplantation is essential to minimize graft loss and avoid retransplantation (9)

Another important indication is cholestatic disease (13%), it contains for example primary biliary cirrhosis(PBC), biliary atresia or primary sclerosing cholangitis(PSC). Followed by primary liver tumors (10%) and acute hepatic failure (9%) (4)

The indications for treating liver-cancer with a transplantation have changed dramatically over the last decade with reduction for cancers other than hepatocellular carcinoma (HCC). For HCC, the indications are limited by number and size of tumors and missing of portal invasion. This led to better results during the last years (6).

There are also other indications like secondary liver tumors, metabolic diseases (e.g. familial amyloidotic polyneuropathy, wilson's disease, α 1-antitrypsin deficiency, and others), Budd – Chiari, benign liver tumors or polycystic disease and parasitic disease, but they do not occur so often (4).

Furthermore, there are certain contraindications to transplantation. Absolute contraindications include extrahepatic malignancy, irreversible brain damage, active drug or alcohol abuse (10), and also severe cardiopulmonary or other comorbid conditions, active and uncontrolled infections outside the hepatobiliary system, hepatic malignancy with diffuse or macrovascular tumor invasion and psychosocial factors that would inhibit recovery after transplantation (5).

Relative contraindications are age, cholangiocarcinoma, chronic or refractory infections, portal vein thrombosis, human immunodeficiency virus (HIV), previous malignancy or poor social support (11).

1.1.1.2 Heart transplantation

Heart transplantation (HTX) is the treatment of first choice for patients with end-stage heart failure, where all other treatments possible are exhausted. (12)

Due to the shortage of donors, certain limitations have been placed to restrict transplantation to those patients, who have the greatest benefit from transplantation (10).

Also due to the lack of donors, left ventricular assist devices (LVAD) are being used increasingly to treat end-stage heart failure and to bridge the time to transplantation (13).

Patients who should be considered for HTX (10):

1. Patients with a systolic heart failure (with an ejection fraction under 35%), with an etiology, that is ischemic, dilated, valvular, hypertensive or others, and not because of an amyloid etiology, HIV or cardiac sarcoma.
2. Ischemic heart disease with angina pectoris, which is difficult to manage, and unsuccessful other therapies (like coronary artery bypass or percutaneous revascularization, maximal tolerated medical therapy, direct myocardial revascularization or transmyocardial revascularization)
3. Unmanageable arrhythmia, not got under control by a pacing cardioverter – defibrillator, and not amenable to electrophysiology single or combination medical therapy; and also not eligible for ablative therapy.
4. Hypertrophic cardiomyopathy, when the New York Heart Association (NYHA) IV symptoms remain in spite of maximal therapy (like alcohol injection, myomectomy, mitral valve replacement, maximal medical therapy or pacemaker therapy).
5. Congenital heart disease, without the complication of fixed pulmonary hypertension.

There are also contraindications to HTX. Patients should not be older than 65 to 70 years (according to the centre of transplantation). They must not have diabetes mellitus with end–organ damaging, or a fixed pulmonary hypertension with a vascular resistance bigger than 6 wood units. Neither an active infection nor sepsis, and also no HIV infection may occur. Further contraindications are

irreversible hepatic or renal dysfunction, active malignancy or a peripheral vascular disease. Moreover it is worthy to mention, that a recent substance abuse or poor compliance are reasons to abandon transplantation (14)

Besides, psychosocial impairment, that harms the transplanted heart (like medication noncompliance or antisocial personality disorder), is a contraindication (10).

1.1.2 Surgery

1.1.2.1 Liver Transplantation

Brain-dead organ donors with normal liver function generally are accepted as donors for LTX. Due to the constant increase of patients on the waiting list, organs with extended donor criteria are accepted increasingly (15). The existence of HIV and malignancy are still absolute contraindications (6).

The first orthotopic LTX was performed by T.E Starzl in 1963 (16). This technique required the total disconnection of the caval bloodflow by clamping the supra- and infrahepatic inferior vena cava. The removal of the recipients liver happened after transaction of the retrohepatic vena cava above and below the liver (17). The disadvantages of this technique were serious hemodynamic instability and massive decrease of the cardiac preload (16).

To avoid these handicaps, the use of venous – venous bypass was introduced . But to do this, systemic heparinisation was necessary, and that led to massive operative bleeding, which was difficult to handle. Bypass – systems got first in clinical practice, as the tubes were coated with heparin. Nevertheless there were some complications due to the bypass, like air embolisation, inguinal and axillar seroms and infections, or thrombosis (15).

Later, the so-called “piggyback” technique was invented. Here the vena cava and so the bloodflow were persisting during hepatectomy (18).

With optimal cell protection the time of ischemia can be widened up to 12 (-16) hours, certainly it should be held as short as possible. The transplantation takes place conventional or with modification of the caval anastomosis (so called “piggyback” technique) (15). The “Piggyback” technique lowers the risk of injury to the adrenal vein because no retrocaval resection is needed, and furthermore instead of two end-to-end anastomoses only one cavo-caval anastomosis is necessary (19).

1.1.2.1.1 Surgical technique

The abdomen is opened with lateral upper abdomen laparotomy to the right with median extension to the xyphoid (inverted L incision), or if there is a very huge liver, the incision is extended to the left additionally (15).

First there is the ligation and division of the common bile duct and hepatic artery branches and skeletonization of the portal vein. After this the right liver lobe is reflected to the left for the purpose of exposing the right and anterior sides of the inferior vena cava (IVC) (18).

Then the hepatocaval ligament is divided, which is a key point, especially when the prominent part of the caudate lobe encircles the IVC. All the smaller hepatic veins, which drain the caudate lobe and right accessory veins are ligated from below upwards selectively. So the suprahepatic veins are approached. Moreover the right hepatic vein is transected and oversewn. This facilitates the safe isolation of the middle and left hepatic veins. Next the portal vein is clamped and the caudate lobe is dissected. Now the middle and left hepatic veins can be exposed and transected after application of the vascular clamp (20). Then the recipients liver can be removed and the graft can be implanted (15).

On the backtable the caudal end of the donor IVC is shortened and closed over a silastic tube for venting purpose with a purse string suture around. Also the suprahepatic IVC is left open and the opening is extended by a longitudinal midline incision on the posterior wall (18).

The first step of implantation is the suprahepatic anastomosis between the donors' suprahepatic vena cava and a vascular cuff formed by the lumen of the middle and left hepatic vein. Next the portal vein anastomosis is done end-to-end and the liver is flushed with blood (20). The arterial anastomosis is performed in end-to-end technique, mostly using branch patches on donor as well as recipient side (18). The biliary tract reconstruction is done in end-to-end choledocostomy. A T-tube stent is not used routinely anymore, to prevent biliary tract complications (21).

1.1.2.2 Heart Transplantation

HTX was performed successfully for the first time in 1967, and until 1980 fewer than 360 HTX were performed worldwide (22).

But then it became an established procedure to treat end stage heart failure (23).

Since its first description by Lower and Shumway, the standard technique of orthotopic HTX has remained unchanged (22)

1.1.2.2.1 Surgical technique

First there is a median sternotomy and a vertical pericardiotomy, then the patient is heparinised and prepared for the cardiopulmonary bypass (24).

For the bypass the superior and inferior vena cava, and the ascending aorta are cannulated (25).

After umbilical tape snares are passed around the caval veins, the bypass is initiated, the patient is cooled to 28-30°C, the caval snares are tightened, and the ascending aorta is cross-clamped. It follows the transection of the great vessels above the semilunar commissures, and also the vestibules are incised along the atrioventricular grooves leaving cuffs for the allograft implantation (24).

Then cardioectomy follows. The implantation of the donors heart begins with the anastomosis of the left atrium with continuous suture. When the left atrial anastomosis is finished, the right atrium of the donors heart is cut open from the inferior vena cava to the right heart auricle, and then it is sewed to the atrium of the recipient (25).

Now the pulmonary artery anastomosis is performed end-to-end. Before the aortic anastomosis is also done in end-to-end fashion, the rewarming is induced. Afterwards routine de-airing techniques are employed and the aortic-cross clamp is removed. About half of the transplanted need electrical defibrillation. Then the by-pass and the canules can be removed. After placing temporary epicardial pacing wires in the donor right atrium and ventricle, the implantation is finished (24).

1.1.3 Complications

1.1.3.1 Liver Transplantation

Results after LTX have advanced, with more patients surviving for a longer time. But, contrary to the decrease of early mortality, the amount of later deaths has not improved. There are many reasons for morbidity and mortality, comprising recurrence of the primal disease or side-effects of the immunosuppression. For this reason transplant clinicians have to be conscious, and prevent these complications or treat them in the right way (26).

The primary function of the transplanted organ is essential for the survival of the recipient. When there is a primary non-function of the organ, which can appear with an incidence of 1 – 5%, retransplantation is the only option for treatment. A slow and delayed start of the liver function (primary poor function) is often associated with other organ dysfunctions, such as renal failure, and high infection risk. Especially infections are serious complications in the early phase, and represent the major cause of death after LTX (27).

With the currently used immunosuppressive regime, acute rejection reaction occurs with a prevalence of 10 – 45%. Acute rejection reactions are asymptomatic. To substantiate suspicion, high liver enzymes are important (GPT (glutamic-pyruvic transaminase) > GOT(glutamic-oxaloacetic transaminase)), but evidentiary is only histology. If the rejection reaction occurs later, it is mostly a sign of low immunosuppression, for example when there is less resorption because of gastrointestinal infections, or interaction of the immunosuppressives with other drugs (28).

There is also the chronic rejection reaction, which often proceeds in a prognostic disadvantageous way. But the incidence has decreased in the last years, because of new, more potent immunosuppressants (27).

Complications of the bile duct, such as leaks, narrowing of the anastomosis or intrahepatic strictures, appear in the first months after LTX with an incidence of 10–30%. In most of this cases retransplantation is the only effective treatment (29).

In the first months after transplantation the calcineurin inhibitors (Cyclosporine or Tacrolimus) can lead to fatal nephro- and neurotoxicity. Especially in patients with

restricted renal function prior to transplantation, as for the hepatorenal syndrome or in older people, the immunosuppression has to be adjusted carefully. The neurological adverse effect presents from headache, peripheral polyneuropathy to generalized attacks to pontine myelinolysis (28).

The most common adverse effects of the immunosuppressives, affect the chronic nephrotoxicity of the calcineurin inhibitors, which is associated with irreversible nephrosclerosis and can lead to terminal renal failure. Furthermore, metabolic complications as well as disorders in the bone metabolism can occur (27).

30 – 40% of the recipients develop a restricted renal function, 10 – 20% insulin dependent diabetes mellitus, and 20 – 40% hyperlipidaemia (28).

50% of the liver transplanted persons come up with arterial hypertension. Therefore the cardiovascular risk rises seriously. Cardiovascular complications are the most common cause of death in long term progress after LTX.

De novo malignancies are seen up to 20 times more frequent in liver transplanted patients than in the normal population. Usually the tumors are diagnosed in an advanced stadium, so curative options of therapy are often not possible. So periodic screening, like x-ray of the chest, PSA controls with urological examination, mammography, coloscopy, are essential (28).

1.1.3.2 Heart Transplantation

Heart allograft rejection is the regular host response to cells identified as nonself. Most cases are cellular-mediated by macrophages, cytokines and T-lymphocytes. Humoral-mediated rejection (vascular rejection) is not so common. About 80% of acute rejections occur in the first three months after HTX (24).

The most common risk factors are human leukocyte antigen (HLA) mismatches, female gender and allografts from younger or female donors (14).

Rejection is still a major cause of morbidity, though most of the episodes can be reversed with corticosteroids (30)

In the time before Cyclosporine, the common manifestations of acute rejection were fever, malaise, leucocytosis, pericardial friction rub, low cardiac output, arrhythmia and signs of congestive heart failure. In the era of Cyclosporine, patients can stay asymptomatic, even in late stages of rejection (24).

Due to this, early detection is very important to minimize the injury of the graft. The golden standard of detection remains biopsy of the right ventricular endomyocard (24).

The most important causes of mortality and morbidity are infections. Patients who are transplanted, often do not occur with the typical signs and symptoms of infection because of their immunodeficiency (31).

Infections can be differentiated in three time periods: early post-transplantation period (first month), between one and six months and late post-transplantation period (over six months) (30).

The early post-transplantation period can be seen as the nosocomial phase. Most infections in this time occur because of catheter insertion and incision area; nosocomial pneumonia is dominant. The major bacterial agents are gram-negative and staphylococcus bacteria. The most common viral agent is herpes simplex virus (HSV) (31).

Classical infections related to transplantation usually are seen between the first and the sixth months. Patients are vulnerable for infections with reactivated viruses like HSV, Epstein-Barr virus (EBV) and most prominent CMV. Also opportunistic infections such as pneumocystis carinii pneumonia and infections due to Nocardia and Aspergillus may occur. Furthermore latent infections can reactivate, like mycobacterium tuberculosis. Later (after six months) HTX patients

are at risk for community-acquired pathogens. A few continue to be affected with chronic infections like hepatitis B (HBV) and C (HCV) or CMV (30).

About 95% of patients are affected by hypertension five years after transplantation. This fact depends mostly on calcineurin inhibitors and corticosteroid therapy (32).

Moreover, chronic renal insufficiency is an important complication of HTX. The main risk factor for developing renal failure is the use of calcineurin inhibitors in the immunosuppressive regime (30).

Cardiac transplanted persons are also at risk of to develop diabetes mellitus, because of the medication with corticosteroids and calcineurin inhibitors (31).

Many patients suffer from hyperlipidemia after transplantation. The main causes for this are inappropriate nutrition, genetic predisposition and immunosuppressive therapy (30).

Another important morbidity factor is osteoporosis. 20% of the transplanted persons have severe osteoporosis on the neck of femur and 28% on lumbar vertebrae, two years after transplantation (24).

A major role as a cause of death after HTX are malignancies. The long term risk of malignancies after cardiac transplantation is 1-2% per year. Compared with an age matched control population, this risk is 10 to 100 times higher. The most common types are malignant tumours of the skin and lymphomas (30).

Gastrointestinal complications, like peptic ulcers, pancreatitis, intestinal perforation and bleeding, are seen in 15 – 30% of transplanted in the first 3-5 years (24).

Due to the side effects of steroids some patients develop ocular complications, such as glaucoma and cataract. Also retinitis can occur, mostly because of CMV infection (30).

Despite the advances in long term outcome of HTX, the major cause of mortality is still cardiac allograft vasculopathy, with about 20% of all deaths after the first year following transplantation (33). It can begin soon after transplantation developing rapidly in a few months. Its incidence is about 10 – 15% and is seen in about half the patients by five years after cardiac transplantation.

Cardiac allograft vasculopathy is a complex interaction between immunologic and non-immunologic factors which leads to repeating endothelial injury and inflammatory response. This is followed by intimal hyperplasia and proliferation of vascular smooth muscle cells. The intramyocardial and epicardial coronary

arteries become diffuse and concentric thickened. This process can progress to an almost complete occlusion of the coronary arteries, leading to micro and macro infarctions. Predisposing factors include immunological mechanism with alloreactive T-cells and the humoral immune system, and non immunologic factors like infections (especially the CMV), older donor age, hypertension and injury because of ischemia or reperfusion. Usually patients are asymptomatic, so diagnostic is very important. Annual coronary angiography is done for diagnostic and surveillance of cardiac allograft vasculopathy, but a more sensitive tool for early disease stages is intravascular ultrasound (34).

1.2 Cytomegalovirus

1.2.1 General considerations

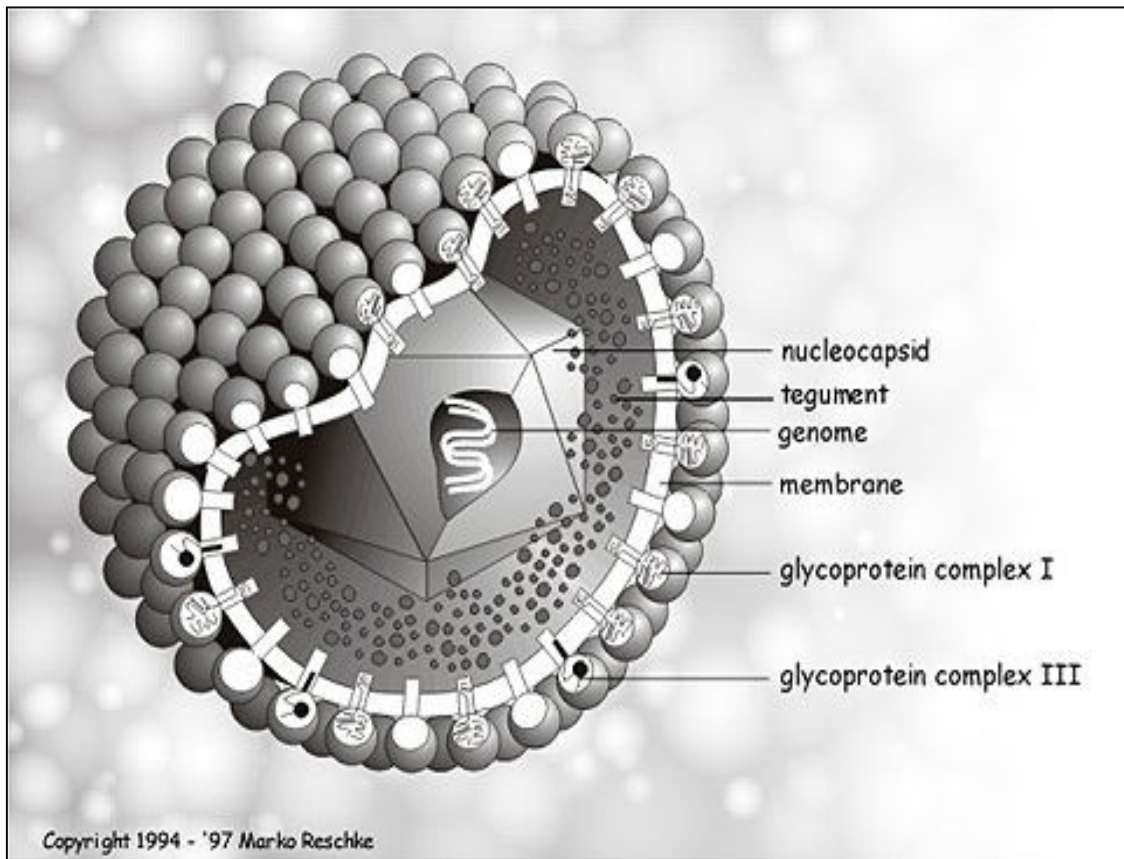


Figure 1: Human cytomegalovirus (35)

The cytomegalovirus (CMV) belongs to the family of the herpesviridae, which includes HSV, varicella-zoster virus (VZV), EBV and herpesvirus 6, 7 and 8. Its subfamily are the beta – herpesviridae, and its genus is human herpesvirus 5. CMV is the largest discovered human herpesvirus, with a genome of approximate 260 kbp (35).

It has a double – stranded linear DNA, coated by a proteinaceous matrix, the tegument, encircled by a lipid doublelayer, which encloses viral glycoproteins (36).

CMV can be transmitted in different ways. There is the transmission via sexual contact, blood transfusion, saliva, placental transfer, breast feeding, haematopoietic stem cell – transplantation and solid organ transplantation (37).

1.2.2 Clinical effects

There are direct and indirect effects of CMV infection. The direct effects demonstrate as CMV syndrome or as tissue-invasive disease (38).

The CMV syndrome shows flu-like and mononucleosis-like symptoms, frequently with neutropenia (39).

Tissue – invasive disease may occur as nephritis, hepatitis, carditis, pneumonitis, pancreatitis, colitis or retinitis. There is a preference for the greatest inflammation inside the transplanted organ, i.e. liver transplanted patients tend to hepatitis, heart transplanted patients to carditis (38). The tissue-invasive CMV disease often affects also the gastrointestinal tract in form of CMV gastritis, esophagitis, enteritis and colitis (39).

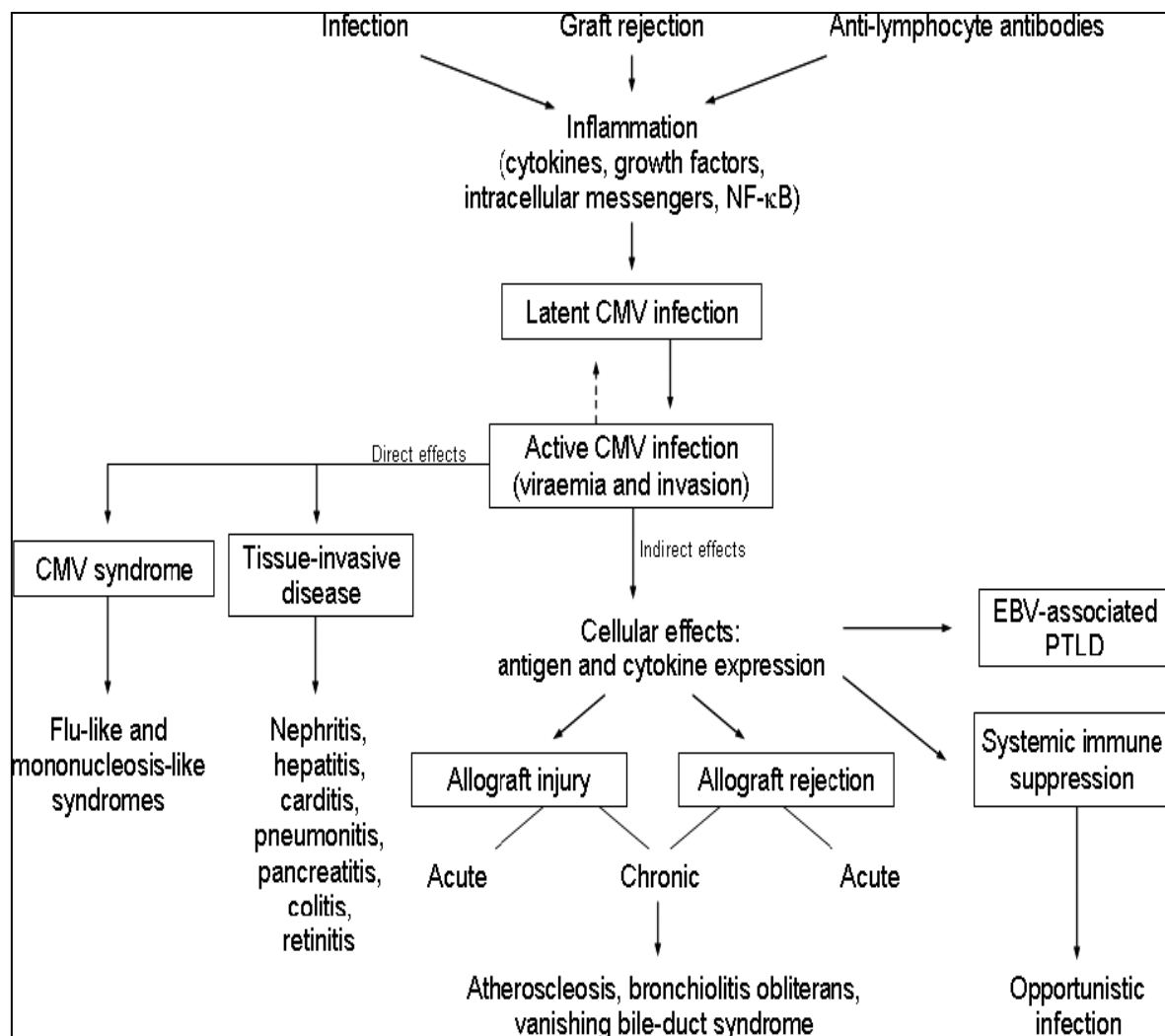


Figure 2: Overview of cytomegalovirus (CMV) infection: direct and indirect effects (38)

Before there was an appropriate therapy for CMV-infection available, a disseminated tissue-invasive CMV infection often was mortal (38).

The destruction of the tissue, which is infected, is a direct consequence of viral replication and cell lysis, linked with host immune responses leading to destruction of tissues presenting CMV antigens on cell surfaces. The chance to develop CMV disease increases logarithmically with viral load (40).

Besides the direct effects of CMV-infection, there are also indirect effects. This term is used for those effects, which are independent of high level CMV viremia. The indirect effects are rather a function of immune responses in the setting of longer periods of low level viral replication. These effects are diverse and may contain increased graft rejection with manifestations such as vanishing bile-duct syndrome following LTX or accelerated coronary atherosclerosis following HTX, secondary bacterial and fungal infections, development of cancer, development of post-transplant diabetes mellitus, and decreased graft and patient survival (38) (41).

1.2.3 Diagnostic

Serology: The production of CMV-specific antibodies is the manifestation of the humoral response to CMV infection. Immunoglobulin M (IgM) antibodies against CMV are produced early (2 to 4 weeks after primary infection), and IgG antibodies occur after this. In transplantation centres CMV serology is not indicated for diagnostic of CMV disease, because CMV infection is widespread and therefore many adults are seropositive (IgG) for CMV (42), therefore CMV serology is used in identifying the organ donors and recipients, who have been infected previously. It is important to estimate the risk of CMV reactivation and disease. When there is a seropositive donor for a seronegative recipient (D+/R-), the recipient has the highest risk to develop CMV disease after transplantation; D-/R- have the lowest risk of reactivation of CMV (39).

Viral cultures: Traditionally replicating CMV by cell culture has been the standard method in the diagnostic of CMV infection. The proof of CMV in cell cultures is highly correlated with CMV disease, but the low sensitivity limits its value. Moreover it is time-consuming and elaborate (36).

Antigenemia assays: This is a fast quantitative method to detect CMV antigens by directly immunostaining polymorphonuclear leukocytes (PNM) from blood samplings with mononuclear antibodies direct against the CMV lower-matrix protein pp65 (UL83). Antigenemia assays are not dependent on the presence of replicating virus and they are more sensitive than the cell cultures. They are also highly specific for CMV; but the result is better interpreted in quantitative wise to diagnose and predict CMV infection (that means a higher level of antigenemia correlates with active disease). Many transplantation centres have accepted the use of this assays as guiding to the use of preemptive therapy (42).

Nucleic acid amplification: The widely spread use of polymerase chain reaction (PCR) technology to detect viral nucleic acid (DNA or RNA) is the most important innovation in laboratory diagnostic and clinical management of CMV infection after transplantation. Many centres use in house CMV PCR testing with common thermocycling instruments programmed for nucleic acid amplification for forty to forty-five cycles followed by gel electrophoresis of the PCR-amplified products and probe hybridization techniques. The assay is cumbersome and lasts long. It is highly sensitive but the predictive value is low. Moreover the techniques used in the different centres are diverse, and so results are not reproducible among diverse laboratories (42) (43).

The recent disposability of automated PCR instruments that combine rapid thermocycling formats with the capacity for real time quantification of viral genomes has changed the practice of CMV diagnostic. The assays are standardized, and so reproducible among many centres. The technique is also much more timesaving (42).

Nucleic acid hybridization: The Digene Hybrid Capture CMV DNA assay (Digene Corporation, Silver Spring, Md.) is a qualitative, fast, signal-amplified solution hybridization assay using RNA samples that bind to the DNA target followed by antibodies directed to RNA-DNA hybrids, and also a sensitive chemoluminescence detection system. It is more sensitive than cell cultures, and is similar sensitive and specific like antigenemia. But due to the qualitative nature of this assay, the clinical significance of a positive result is not clear, because the assay could detect subclinical CMV replication that may not emerge into clinical disease (42).

1.2.4 Prevention

Due to the adverse effects of CMV infection on transplant outcome, prevention is important for the management of such patients.

There are two main strategies for the prevention of CMV disease:

- **Preemptive therapy:** CMV reactivation is monitored aggressively by sensitive assays and upon detection, the antiviral therapy is applied preemptively to prevent the progression to clinical disease. The most important thing in preemptive therapy is to detect the presence of CMV reactivation before the onset of clinical symptoms, so antiviral drugs can be administered early in order to stop progression to clinical disease. The outcome of this therapy is highly related to the patients compliance with CMV surveillance, very sensitive assays to predict the risk of developing CMV disease and early administration antiviral drugs, like intravenous (IV) or oral Ganciclovir or Valganciclovir (39).

- **Antiviral prophylaxis:** Antiviral drugs are administered to patients, who are at risk to develop CMV disease.

A number of clinical trials have shown that antiviral prophylaxis prevents highly effective the direct, and maybe the indirect effects of CMV (44).

- The application of *Acyclovir* as anti-CMV prophylaxis after transplantation has been replaced by Ganciclovir and Valganciclovir, because of their better effectivity in preventing CMV (39).
- *Ganciclovir*: It is a nucleoside analogue, which is monophosphorylated in infected cells by the gene product of UL97. It is also a competitive inhibitor of the DNA polymerase UL54 (35).

The present data suggests, that Ganciclovir-based treatment is more effective (in comparison with Acyclovir and immunoglobulins) in lowering the incidence of CMV infection after transplantation. One study showed, that the use of IV Ganciclovir for ninety to hundred days reduced the incidence of CMV disease in high risk (D+/R-) liver transplant recipients

to 5,4% (compared to 40% in patients receiving less than seven weeks of prophylaxis) (45). A disadvantage of IV Ganciclovir was the need for longterm IV access, and so the risk of phlebitis, thrombosis and infusion associated infections. So oral Ganciclovir was made available, but its absorption is bad, and therefore the systemic Ganciclovir levels are low (39).

- *Valganciclovir*: It is a valine ester of Ganciclovir, and therefore its absorption is better, with systemic drug levels comparable with IV Ganciclovir. It is shown by pharmacokinetic studies that a 900 mg dose of Valganciclovir contains a similar systemic concentration as IV Ganciclovir with a dose of 5 mg/kg (46). Valganciclovir is the most used drug in preventing CMV disease after LTX (39).
- *Intravenous immunoglobulin*: It supplies passive humoral immunity against CMV. The results of a randomized study of prophylaxis showed a decrease in CMV-related syndromes in immunoglobulin recipients after renal transplantation. The combination of immunoglobulin and Ganciclovir is gold standard for CMV-associated pneumonitis (39).

Both options are highly effective in preventing CMV infection after transplantation (39). But in general antiviral prophylaxis is regarded as a more effective procedure and is exercised by most of the transplant centres in preventing primary CMV infection in high risk recipients (D+/R-) (47).

Also the American Society of Transplantation recommends the use of antiviral prophylaxis in all CMV D+/R- solid organ transplant recipients (48).

Besides, primary antiviral prophylaxis has the added effect, that it reduces fungal and bacterial opportunistic infections and mortality (49).

1.2.5 Therapy

At the moment, the recommendation for antiviral treatment of CMV disease after transplantation is IV Ganciclovir. Moreover, it is very important to reduce the degree of immunosuppression. Oral Ganciclovir should not be administered to treat active CMV disease, because of its low bioavailability. But Valganciclovir provides high systemic concentration of Ganciclovir, so oral treatment of CMV disease is possible now. The continuance of treating the CMV disease should be individualized. The persistence of CMV at the end of the therapy (detected by PCR or pp65 antigenemia) is associated with high risk of relapse. It is generally approved, that multiple (not less than two) weekly negative CMV PCR results should be obtained, before antiviral treatment is stopped (39).

1.3 mTor Inhibitors

1.3.1 General Considerations

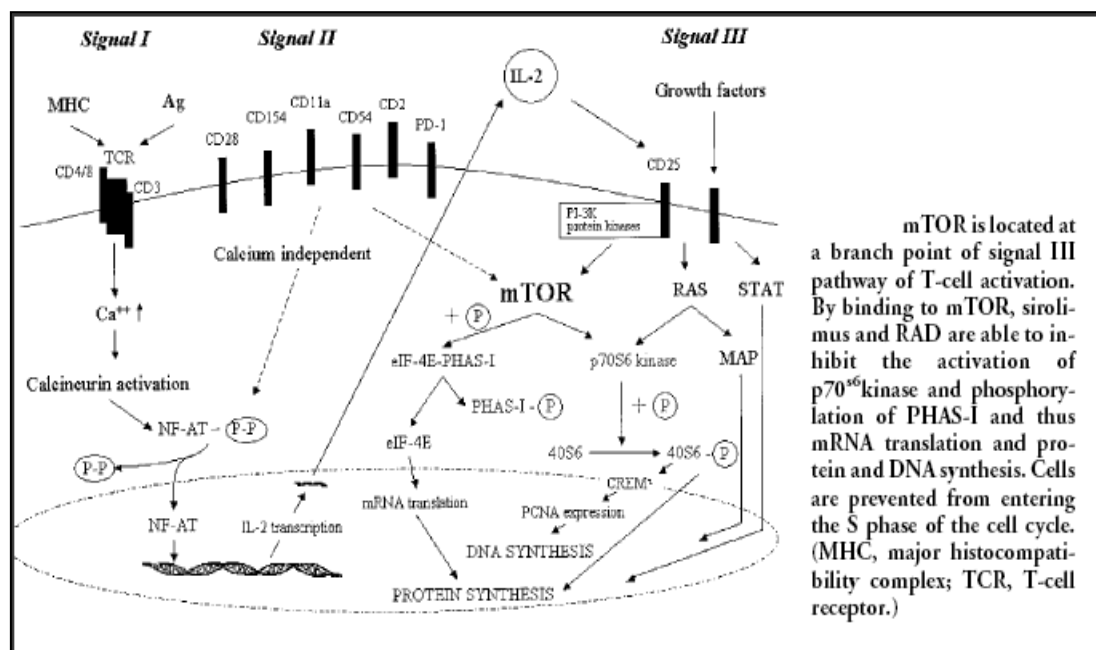


Figure 3: mTOR pathway (50)

The relatively new class of immunosuppressive drugs are the inhibitors of the mammalian target of rapamycin, the so called mTor inhibitors (50).

Sirolimus, the first substance of this group, was isolated from a fungus, called *Streptomyces hygroscopicus*, collected in the soil of Rapa Nui (51).

Primary it was found to have antifungal and antitumor potency. Later it was detected that it has potent antiproliferative activity. Afterwards the mechanism of action was understood (50).

1.3.2 Mechanism of action

Like Tacrolimus and Cyclosporine, mTor inhibitors are usefull therapeutic drugs for studying T-cell signal transduction (52).

mTor inhibitors interact with a family of intracellular binding proteins called FKBP (FK binding proteins; the most relevant is FKBP12, a peptidyl isomerase), and build an immunophilin complex (51).

The target of this complex is the multifunctional serine-threonine kinase of the phosphatidylinositol-3-kinase (PI3K) pathway, which is called the mammalian target of rapamycin (mTOR) (53)

mTOR sits at the branchpoint of the signal 3 pathway in activated T cells (54).

When activated, mTOR transduces signals that trigger the synthesis of ribosomal proteins, translation of a particular subset of messenger RNA (mRNA) transcripts, and the accumulation of cyclin-dependant kinases, advancing the progression of the cell cycle. This leads to activation and proliferation of T and B cells and production of antibodies, and also proliferation of non-immune cells like fibroblasts, hepatocytes and smooth muscle cells (50).

mTOR is known to have two main functions:

- Activation of p70^{S6} kinase. It plays a key role in signal transduction resulting to DNA synthesis (55).
- Activation of the eukaryotic initiation factor 4E (eIF-4E)-phosphorylatable heat stable protein 1 (PHAS 1) pathway, which is more involved in protein synthesis (50).

By binding and inhibiting mTOR, Everolimus and Sirolimus inhibit the activation of p70^{S6} kinase leading to stop the cell cycle at the G₁ to S phase (signal III pathway). Unlike the calcineurin inhibitors, the mTOR inhibitors block growth factor driven proliferation of hematopoietic and non-hematopoietic cells, including bronchial and vascular smooth muscle cells (55).

Indirectly the mTOR inhibitors block effector function of CD4⁺ helper cells and CD8⁺ T cells, monocyte activation, and B cell proliferation and differentiation (50). Because apoptosis is independent of TOR activity, the mTOR inhibitors preserve apoptosis of T cells (55).

1.3.3 Sirolimus (Rapamune® Wyeth Pharma GmbH)

The lipophilic macrolide Sirolimus, which is a natural fermentation product of *streptomyces hygroscopius*, has the chemical formula $C_{51}H_{79}NO_{13}$ and a molecular weight of 914,2 (50).

Its oral bioavailability is approximately 14% with a time to peak concentration around 1 hour after dosing (52).

The terminal half time of Sirolimus is about 62 hours in stable transplant recipients, and its steady state is usually reached within seven to fourteen days, what makes once daily dosing possible (50).

Sirolimus is metabolized by liver and intestinal cytochrome P-450 enzyme CYP-3A4. The metabolites are eliminated by the gastrointestinal tract (91%) and the kidneys (2%) (52).

Sirolimus has an affinity for FKBP12 that is 3,3 times greater than that of Everolimus (56).

1.3.3.1 Positive effects

In contrast to the calcineurin inhibitors and steroids, Sirolimus alone does not have common side effects, such as nephrotoxicity, neurotoxicity, diabetes mellitus and hypertension (53). In LTX it is reported, that liver transplant recipients using Sirolimus as part of a primary immunosuppressive regimen, the appearance of acute cellular rejection is relatively low, compared to other immunosuppressive drugs (57). Sirolimus can avoid acute graft rejection effectively and prevent refractory acute graft rejection in heart transplanted persons without adverse effects on renal function (58). It is suggested that the synergistic combination of Sirolimus with Cyclosporine reduces the incidence of rejection episodes significantly, allows Cyclosporine dose reduction, and permits withdrawal of corticosteroids from the immunosuppressive regime in human renal transplanted patients (59). Sirolimus is one of the most efficient anticandida agents known, but the development of Sirolimus as a systemic antifungal drug was not continued because of its potent immunosuppressive activity which is an undesirable quality of an anti-infective agent (51). Sirolimus is associated with a lack of severe

intrinsic nephrotoxicity and has advantageous effects on post-transplantation diabetes mellitus, arterial hypertension induced by calcineurin inhibitors (60).

Sirolimus also has antitumor activity, inhibiting proliferation of transformed cell lines of hepatic, renal, melanocytic, lymphoid, central nervous system, osteoblastic and myogenic origin, and also proliferation of B and T cells transformed by EBV and HTLV (Human T-Lymphotropic Virus)-1 (51). In patients with acute coronary syndrome, Sirolimus eluting stents are associated with beneficial long term outcomes and no excess of late stent thrombosis (61).

1.3.3.2 Adverse effects

Adverse effects that require a specific therapeutic treatment, or can influence the short- and long-term morbidity and mortality and graft survival include hypertriglyceridaemia, hypercholesterolaemia, anaemia, infectious and non-infectious pneumonia, lymphocele formation and impaired wound healing (60). An US multicenter trial showed that Sirolimus treated renal transplant recipients had significantly higher serum creatinine compared to Cyclosporine treated recipients, although having fewer rejections (62). In de novo LTX recipients it was shown, that the use of Sirolimus in combination with Cyclosporine and Tacrolimus involved an increase in hepatic artery thrombosis (63). Minor complications of the therapy with Sirolimus are mild and temporary thrombocytopenia, leukopenia, gastrointestinal adverse effects, mucosal ulceration (60) and skin rashes (64). Another potentially serious complication which is associated with Sirolimus therapy is pulmonary toxicity. This means pulmonary symptoms in the absence of infectious causes and other alternative pulmonary diseases, which improve clinically and radiologically after Sirolimus medication is stopped (65). Sirolimus can also cause damaged grafts to leak more protein (64).

A study found out that kidney transplant recipients, when switched from calcineurin inhibitors to Sirolimus, may develop proteinuria (66).

1.3.4 Everolimus (Certican® Novartis Pharma)

Everolimus is a potent immunosuppressive drug that inhibits growth factor stimulated proliferation of hematopoietic and non-hematopoietic cells, including smooth muscle cells (67). Everolimus is a 40-O-(2-hydroxyethyl)-Sirolimus derivate with a molecular weight of 958,25 (50). The bioavailability of Everolimus is 16%, which is slightly higher than that of Sirolimus (68). The peak blood concentration of Everolimus is reached about in 3 hours (69). It has a half time of approximately 30 hours, therefore it has to be taken twice a day. And its steady state is accomplished after 4 to 7 days (68). Everolimus is metabolized by liver and intestinal cytochrome P450 enzyme CYP-3A4. Their metabolites are eliminated in the gastrointestinal tract by over 90 %, and in the kidneys by under 2% (55).

1.3.4.1 Positive effects

Everolimus reduces the incidence of allograft vasculopathy in cardiac transplantation, which is a major cause of morbidity and mortality in heart transplanted patients. It also reduces the intimal thickening of blood vessels to the graft (70). Everolimus shows, compared to mycophenolate mofetil, equivalent efficacy in reducing the incidence of acute rejection after kidney transplantation. And in HTX, compared to azathioprine, superior efficacy in the incidence of rejection (67). It has shown in experimental studies that smooth muscle cell proliferation and transplant arteriosclerosis can be inhibited (71).

Compared with other immunosuppressants, Everolimus treated patients showed in heart and kidney transplantation, that the incidence of CMV infection was lower (67). When combined with reduced Cyclosporine, Everolimus showed in studies a significantly improved renal function, with no loss of efficacy, when compared to full-dose Cyclosporine regimens (71).

Moreover it was suggested that Everolimus may have potent antitumor effects, especially against EBV-associated B-cell lymphomas (69). It is suggested that Everolimus in combination with the aromatase inhibitor letrozole, have the potential to inhibit the growth and proliferation of hormone-sensitive advanced breast cancer, without pharmacokinetic interactions (72).

In drug eluting stents, Everolimus-eluting stents with bioabsorbable polymer is a method to decrease in-stent neointimal hyperplasia and restenosis, which is safe and efficient (73).

1.3.4.2 Adverse effects

Adverse events due to Everolimus include elevated cholesterol and triglycerides, which responds to treatment, and decreased platelet count (67).

Other side effects found included thrombocytopenia and dose dependent increases in the incidence of infection (69).

Studies found out that Everolimus potentiates the nephrotoxicity of Cyclosporine, so that they suggest to use dosage-controlled Everolimus with reduced Cyclosporine dosage to limit nephrotoxicity while maintaining efficacy of immunosuppression (74).

2 Aim of the study

In our study we wanted to evaluate how the immunosuppression with mTOR inhibitors, either Everolimus (Certican® Novartis Pharma) or Sirolimus (Rapamune® Wyeth Pharma GmbH) affect the appearance or recurrence of CMV infection, and if there is a difference in occurrence of CMV infection whether patients are treated with Everolimus or Sirolimus.

Furthermore, one of our question was, if there is a difference in the development of CMV infection depending if the transplanted organ is a heart or a liver.

Besides we wanted to figure out if the CMV serostatus of the donor or the CMV serostatus of the recipient prior transplantation affects the appearance or recurrence of CMV infection.

3 Methods

Patients:

Patients after orthotopic HTX and after orthotopic LTX, transplanted between 1983 and December 2008 at the Medical University Graz, Austria, Department for Surgery, Division of Transplantation Surgery were included in this study.

Inclusion criteria:

Transplant recipients who received either Everolimus (Certican® Novartis Pharma) or Sirolimus (Rapamune® Wyeth Pharma GmbH) in their immunosuppressive regimen were included in our study.

Exclusion criteria:

Patients who did not receive a mTOR inhibitor (Everolimus or Sirolimus) in their immunosuppressive regimen were excluded from the study. Also patients in which mTOR inhibitors were discontinued till December 2008 were excluded.

Data collection:

We collected our data retrospectively from openMedocs (Medical data documentation used at the Medical University Graz), from patients' charts and from the Eurotransplant database.

Following data were collected: the age of the patients when they were transplanted, how many months after transplantation they received a mTOR inhibitor, how long they achieved a mTOR inhibitor. Moreover we looked for the CMV status of donor and recipient prior to transplantation, if and how often the recipients developed CMV infection in their medical history and how long it lasted till they developed CMV infection. Also data about trough levels of immunosuppression was collected.

Detection of CMV:

Evidence of CMV infection was monitored by the CMV antigenemia pp65 assay.

Statistics:

For data analysis, the Statistical Package for the Social Science (SPSS), version 17 was used. Data are expressed as mean $\pm$ SEM or as absolute numbers and/or percentages. Data were compared by Pearson's chi-square or Fisher's exact test (for qualitative variables) and Student t-test for quantitative variables. In all tests used, a p-value of $< 0,05$ (two way analysis of variance) was considered statistically significant.

4 Results

4.1 *General*

In this retrospective study, all patients who received a mTOR inhibitor as part of their immunosuppressive therapy after transplantation at the Division of Transplantation Surgery, Medical University Graz, were included.

Out of a total of 109 patients (100,0%), 40 patients (36,7%) received a heart transplant and 69 patients (63,3%) a liver graft.

Due to the fact, that post- transplant treatment is not carried out at the Division of Transplantation Surgery, but at the Division of Nephrology and Hemodialysis, patients receiving a kidney were not included in this study. Moreover, combined pancreas- kidney transplantation started only 10 years ago at our department and therefore these patients were not included in this retrospective study.

The mean age of the patient population in our study was 57,75 years $\pm$ 2,05 in HTX recipients, and 52,17 years $\pm$ 1,25 in LTX recipients.

The mean age of the patients when they were transplanted, was for HTX patients 47,65 years $\pm$ 2,15, and for LTX recipients 57,51 years $\pm$ 1,2.

The duration from transplantation to the start with mTOR inhibitors was in the average 73 months $\pm$ 7,83 for HTX recipients, and 23,42 months $\pm$ 5,26 for LTX recipients.

CMV infection occurred in the mean 4,5 months $\pm$ 0,5 after medication with mTOR inhibitors in HTX patients, and in LTX patients 19,05 months $\pm$ 4,38 after medication with a mTOR inhibitor.

HTX recipients	
Mean age (years)	57,75 $\pm$ 2,15
Mean age at transplantation (years)	47,65 $\pm$ 2,05
Mean start with mTOR inhibitors after transplantation (months)	73 $\pm$ 7,83
Mean duration till CMV infection after start with mTOR inhibitors (months)	4,5 $\pm$ 0,5

Table 1: HTX recipients

LTX recipients	
Mean age (years)	57,51 ± 1,2
Mean age at transplantation (years)	52,17 ± 1,25
Mean start with mTOR inhibitors after transplantation (months)	23,42 ± 5,26
Mean duration till CMV infection after start with mTOR inhibitors (months)	19,05 ± 4,38

Table 2: LTX recipients

In those patients who developed CMV infection after mTOR inhibitor medication, the mean age of the patients at transplantation was 52,14 years ±2,18, the mean start of mTOR inhibitor therapy was 11,45 months ±3,39 after transplantation, and in average CMV infection occurred 17,73 months ±4,07 after beginning with mTOR inhibitor immunosuppression.

The organ transplant recipients who did not develop CMV infection after mTOR inhibitor therapy, were in the average 50,1 years ±1,27 when they were transplanted, the mean start of mTOR inhibitor immunosuppression was 49,24 months ±5,86 after transplantation.

CMV infection	
Mean age (years)	56,36 ± 2,13
Mean age at transplantation (years)	52,14 ± 2,18
Mean start with mTOR inhibitors after transplantation (months)	11,45 ± 3,39
Mean duration till CMV infection after start with mTOR inhibitors (months)	17,73 ± 4,07

Figure 4: General outcome of patients who developed CMV infection after start with a mTOR inhibitor

No CMV infection	
Mean age (years)	57,91 ± 1,26
Mean age at transplantation (years)	50,1 ± 1,27
Mean start with mTOR inhibitors after transplantation (months)	49,24 ± 5,86

Figure 5: General outcome of patients who did not develop CMV infection after start with a mTOR inhibitor

CMV infection related to the transplanted organ

In HTX recipients CMV infection occurred in 2 cases, that are 5% of all HTX patients and 9,1% of all patients who developed CMV infection. 38 of the patients who underwent HTX did not get an infection with CMV, which are 95% of all HTX recipients in our study.

From all LTX patients 20 developed CMV infection, that are 29% of all LTX recipients in our study and 90,9% of all patients who developed an infection with CMV. In 49 of the patients after LTX a CMV infection did not occur, that are 71% of all LTX recipients.

That means that LTX patients, who received mTOR inhibitors as a part of their immunosuppressive regimen, developed significantly more often CMV infection than HTX patients treated with mTOR inhibitors. ($p=0,003$).

		HTX	LTX	All
CMV infection after mTOR	quantity	2	20	22
	of CMV infection (%)	9,1	90,9	100
	of transplanted organ (%)	5	29	20,2
No CMV infection after mTOR	quantity	38	49	87
	of transplanted organ (%)	95	71	79,8

Table 3: CMV infection after start with mTOR inhibitors related to the transplanted organ

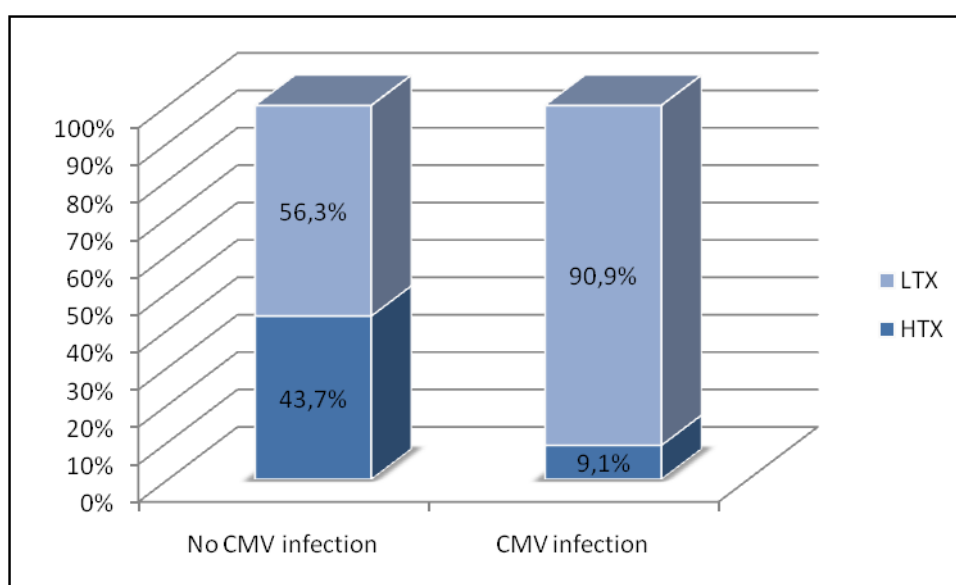


Figure 6: CMV infection after start with mTOR inhibitors depending on the transplanted organ

CMV infection related to the kind of mTOR inhibitor

In those patients who received Everolimus as a part of their immunosuppression, CMV infection appeared in 2 cases which makes 5% of those who received Everolimus, and 9,1% of all patients who developed CMV infection. In 38 of the Everolimus group CMV infection did not occur, that are 95%.

In Sirolimus treated recipients CMV infection occurred in 20 cases which are 29% of Sirolimus treated, and 90,9% of all patients who developed CMV infection. In 49 cases of the Sirolimus group CMV infection did not emerge, that makes 71%.

Viewing to the correlation, significantly more Sirolimus treated patients developed CMV infection than those who received Everolimus. ($p=0,003$).

		Sirolimus	Everolimus	All
CMV infection after mTOR	quantity	20	2	22
	of obtained mTOR inhibitor (%)	29	5	20,2
	of CMV infection (%)	90,9	9,1	100
No CMV infection after mTOR	quantity	49	38	87
	of obtained mTOR inhibitor (%)	71	98	79,8

Table 4: CMV infection related to the obtained mTOR inhibitor

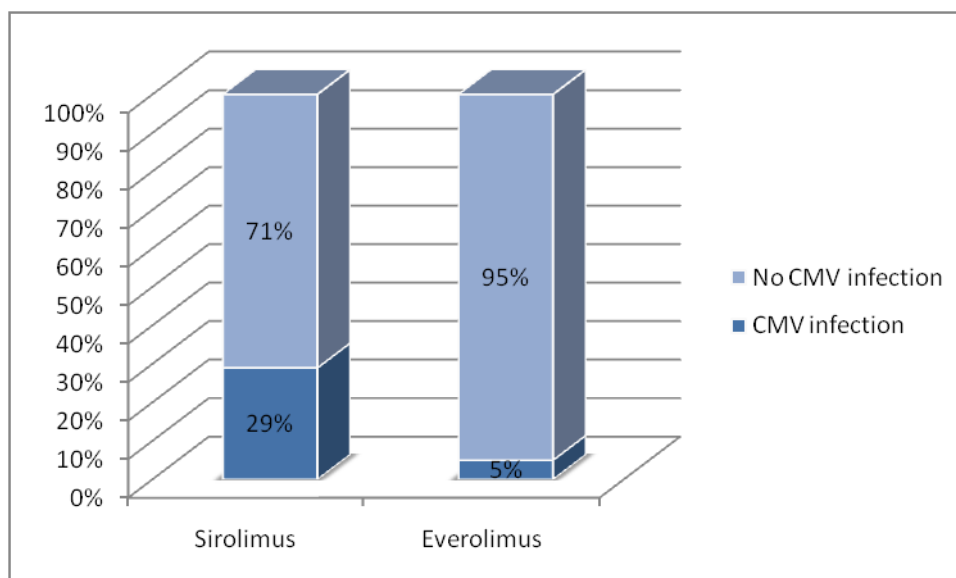


Figure 7: CMV infection related to the obtained mTOR inhibitor

CMV infection related to the CMV status of the donor

When the organ donor was CMV negative, 13 recipients developed CMV infection, which makes 25% of all patients who got an organ from an CMV negative donor, and 59,1% of the patients who developed CMV infection. In 39 recipients CMV infection did not occur, when the donor was CMV negative, that are 75% of all recipients from an CMV negative donor.

9 cases of CMV infections occurred when the organ donor was CMV positive, that are 17,3% of all patients in our study who got an organ from a CMV positive donor, and 40,9% from all patients who got infected by CMV. 43 recipients did not develop infections with CMV when the donor was CMV positive, making 82,7% of the patients who got an organ from a CMV positive donor.

A significant relation between CMV infection and the CMV status of the organ donor could not be shown ($p = 0,472$).

		status of the donor		
		CMV negative	CMV positive	All
CMV infection after mTOR	quantity	13	9	22
	of CMV infection (%)	59,1	40,9	100
	of particular CMV status of the donor (%)	25	17,3	21,2
No CMV infection after mTOR	quantity	39	43	82
	of particular CMV status of the donor (%)	75	82,7	78,8

Table 5: CMV infection after start with mTOR inhibitor related to the CMV status of the donor

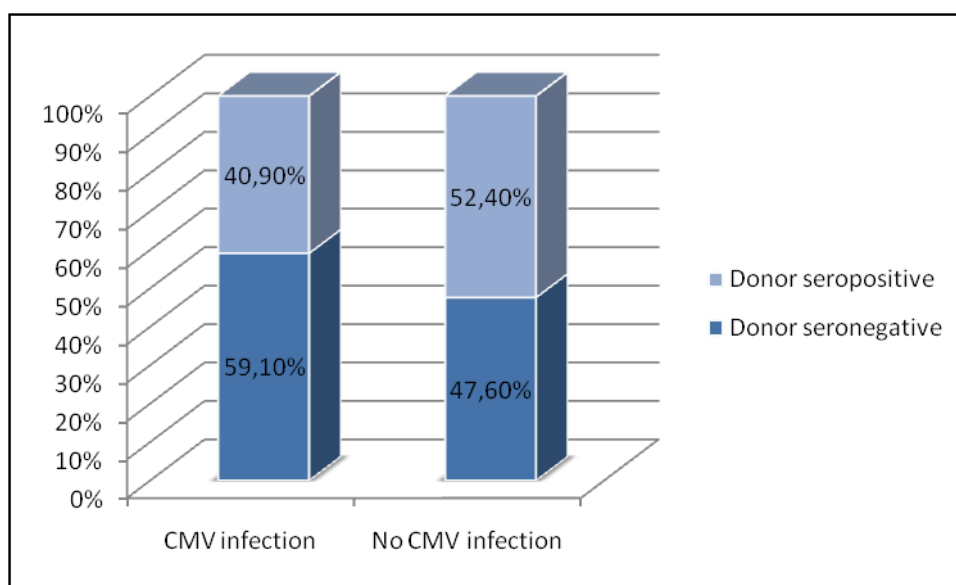


Figure 8: Occurrence of CMV infection after beginning with mTOR inhibitors in relation to the CMV status of the donor

CMV infection related to the CMV status of the recipient

Looking at the CMV status of the recipients in our study we found out that 7 recipients who were CMV negative developed CMV infection, that are 23,3% from the patients with negative CMV status and 36,8% of all patients who got infected by CMV. 23 cases of recipients who had a negative CMV status developed CMV infection which accords to 76,7% of the patients with a negative CMV status.

In 12 persons whose CMV status was positive CMV infection did occur, that are 19% of the recipients where the CMV status was positive and 63,2% of all patients who got infected by CMV. From the organ recipients who showed a positive CMV status 51 did not develop CMV infection, being 81% of the patients with a positive CMV status.

There was no significant relation between CMV status of the recipient and the development of CMV infection after medication with mTOR inhibitors. ($p=0,472$).

		CMV status of the recipient		
		negative	positive	All
CMV infection after mTOR	quantity	7	12	19
	of particular CMV status of the recipient (%)	23,3	19	20,4
	of CMV infection (%)	36,8	63,2	100
No CMV infection after mTOR	quantity	23	51	74
	of particular CMV status of the recipient (%)	76,7	81	79,6

Table 6: CMV infection related to the CMV status of the recipient

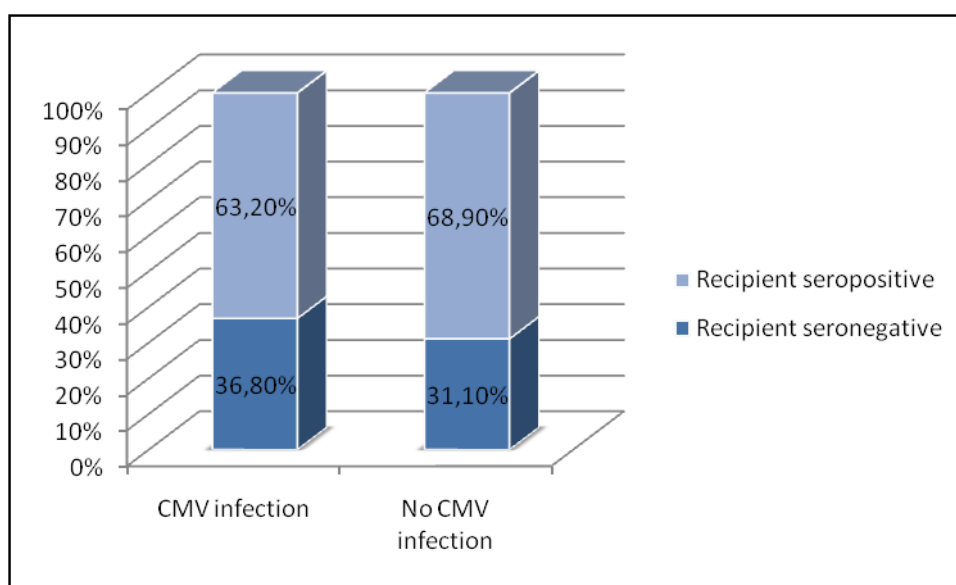


Figure 9: Infection with CMV after start with mTOR inhibitor related to the CMV status of the recipient

Transplanted organ related to the kind of mTOR inhibitor

33 HTX recipients had Everolimus in their immunosuppressive regimen which makes 82,5% of HTX patients and also 82,5% of the Everolimus group. 7 LTX recipients received Everolimus, that are 10,1% of all LTX patients in our study, and 17,5% of the Everolimus group.

Sirolimus was administered to 7 cases of HTX recipients making 17,5% of HTX patients in our study, and 10,1% of the Sirolimus group. We found that 62 patients who underwent LTX had Sirolimus in their immunosuppression, that are 89,9% of the LTX recipients and also 89,9% of the Sirolimus group.

This shows that in HTX recipients Everolimus was significantly more often prescribed than in LTX patients. And in LTX recipients Sirolimus was significantly more often given as part of their immunosuppression than in HTX ones. ($p=0,000$)

		Everolimus	Sirolimus	All
Heart transplantation	quantity	33	7	40
	of heart transplantation (%)	82,5	17,5	100
	of obtained mTOR inhibitor (%)	82,5	10,2	36,7
Liver transplantation	quantity	7	62	69
	of liver transplantation (%)	10,1	89,9	100
	of obtained mTOR inhibitor (%)	17,5	89,9	63,3

Table 7: Transplanted organ related to which mTOR inhibitor was given

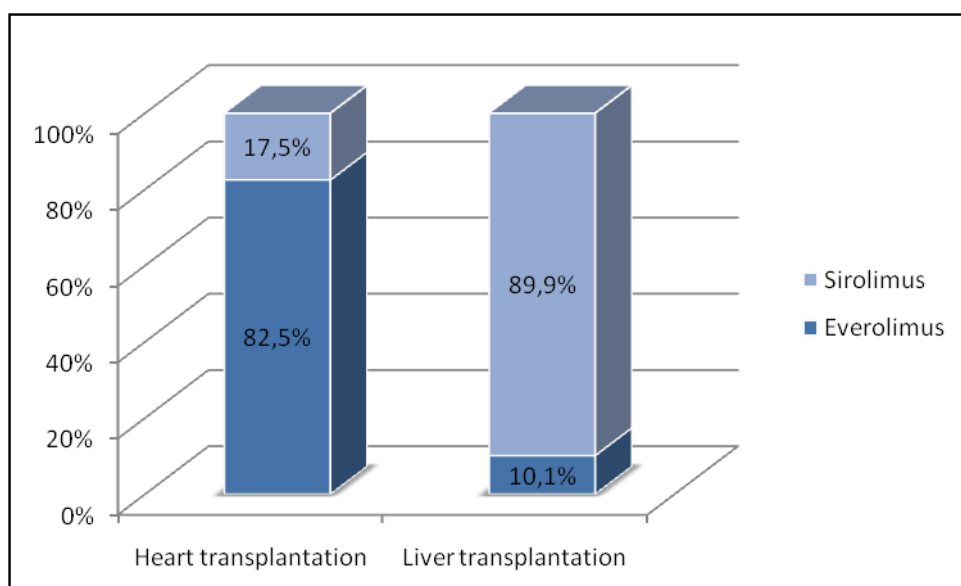


Figure 10: Transplanted organ related to the kind of mTOR inhibitor

Transplanted organ related to the CMV status of the donor

In HTX patients 19 got an organ from a donor with negative CMV status which makes 36,5% of donors with a negative CMV status and 50% of HTX recipients. There were also 19 cases of donors with positive CMV status in HTX patients that are 36,5% of organ donors with a CMV positive status and 50% of the patients who underwent HTX.

33 LTX recipients received an organ from a CMV negative donor making 63,5% of the donors with negative CMV status, and 50% of the LTX patients. The equal amount of patients who underwent LTX got their organ from a CMV positive donor, 33, which makes also 63,5% of CMV positive donors and 50% of the liver transplanted.

There was no significant difference in the CMV status of the donor whether HTX or LTX was performed. ($p=1,000$).

		status of the donor		
		CMV negative	CMV positive	All
Heart transplantation	quantity	19	19	38
	of heart transplantation (%)	50	50	100
	of particular CMV status of the donor (%)	36,5	36,5	
Liver transplantation	quantity	33	33	66
	of liver transplantation (%)	50	50	100
	of particular CMV status of the donor (%)	63,5	63,5	63,5

Table 8: Transplanted organ related to the CMV status of the donor

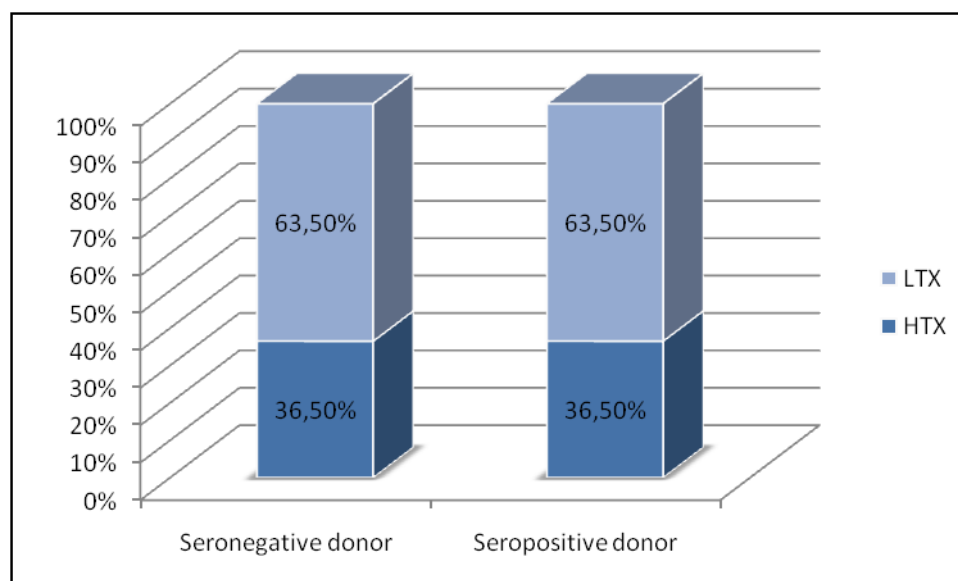


Figure 11: Transplanted organ related to the CMV status of the donor

Transplanted organ related to the CMV status of the recipient

We found that 11 HTX recipients had a negative CMV status equivalent with 29,7% of all HTX patients and 36,7% of all organ recipients with negative CMV status. 26 cases of patients who underwent HTX had a positive CMV status which are 70,3% of HTX recipients and 41,3% of all whose CMV status was positive.

In LTX patients 19 cases had a negative CMV status correspondent with 33,9% of all LTX recipients and 63,3 % of all patients with a negative CMV status. 37 patients who underwent LTX had a CMV status that was positive which coincides with 66,1% of the liver group and 58,7% of patients with positive CMV status.

There was no significant correlation between the CMV status of the recipients and the transplanted organ. ($p=0,821$).

		status of the recipient		
		CMV negative	CMV positive	All
Heart transplantation	quantity	11	26	37
	of heart transplantation (%)	29,7	70,3	100
	of particular CMV status of the recipient (%)	36,7	41,3	39,8
Liver transplantation	quantity	19	37	56
	of liver transplantation (%)	33,9	66,1	100
	of particular CMV status of the recipient (%)	63,3	58,7	60,2

Table 9: Kind of transplantation related to the CMV status of the recipient

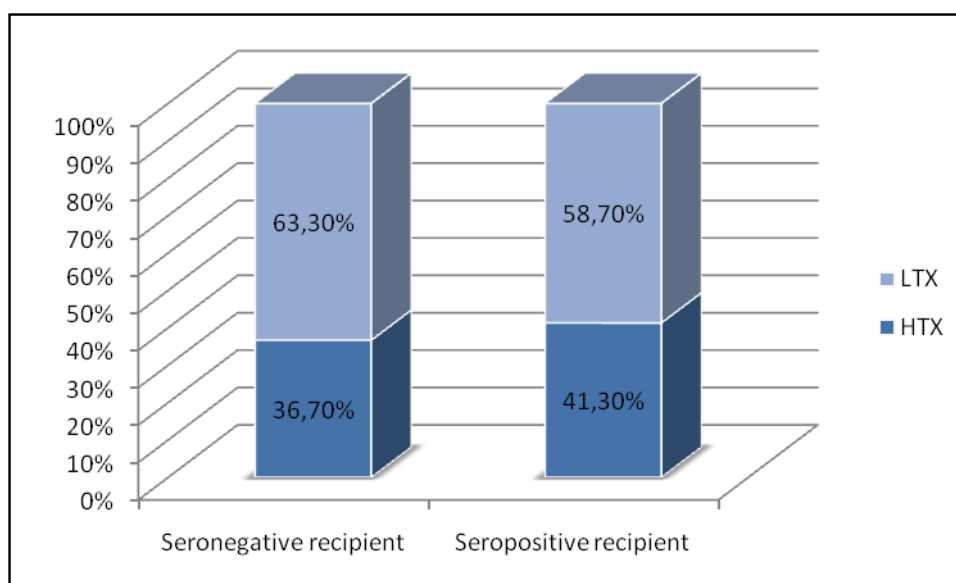


Figure 12: Transplanted organ related to the CMV status of the recipient

4.2 Heart transplantation

CMV infection related to the CMV status of the donor

In those HTX recipients who did not develop CMV infections there were 19 cases whose donor had a negative CMV status correspondent with 100% of the HTX recipients who got their organ from a CMV negative donor, and 52,8% of those recipients who did not develop CMV infection. In 17 recipients who underwent HTX, where CMV infection did not occur the donor was CMV positive, that is 89,5% of HTX recipients getting a heart from a donor with CMV negative status, and 47,2% of those HTX recipients who did not develop CMV infection.

From HTX patients no one got infected by CMV when the organ came from a donor whose CMV status was negative. The only 2 cases in our HTX recipients where CMV infection did occur got their organ from a CMV positive donor, this is equivalent with 100% of heart transplanted infected by CMV, and 10,5 % of those whose donor had a negative CMV status.

A significant relation between CMV infection and the CMV status of the donor could not be shown in HTX recipients. ($p=0,486$).

		status of the donor		
		CMV negative	CMV positive	All
CMV infection after mTOR	quantity	0	2	2
	of CMV infection (%)	0	100	100
	of particular CMV status of the donor (%)	0	10,5	5,3
No CMV infection after mTOR	quantity	19	17	36
	of particular CMV status of the donor (%)	100	89,5	94,7

Table 10: CMV infection in HTX recipients after start with mTOR inhibitors related to the CMV status of the donor

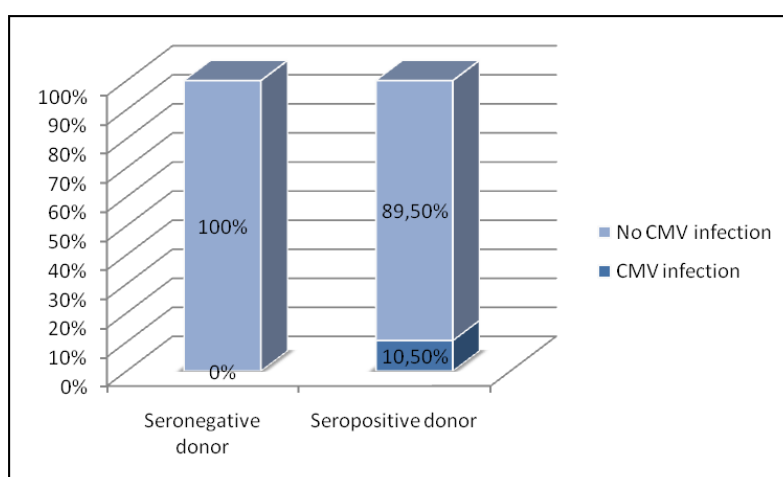


Figure 13: CMV infection in HTX patients related to the CMV status of the donor

CMV infection related to the CMV status of the recipient

Regarding HTX recipients who did not get infected by CMV after their treatment with mTOR inhibitors:

- 11 of them had a CMV status that was negative, correlating with 31,4% of HTX recipients who did not develop CMV infection, and 100% with a negative CMV status.
- In 24 cases the CMV status was positive, that are 68,6% of HTX patients where no CMV infection occurred, and 92,3% of HTX patients with a positive CMV status.

In HTX patients who got infected by CMV, our study found out that:

- None of the recipients who had a negative CMV status developed CMV infection.
- 2 recipients with a positive CMV status developed CMV infection, which makes 100% of CMV infected HTX recipients, and 7,7% of HTX recipients with a positive CMV status.

Our study could not find any significant correlation between CMV infection after start with a mTOR inhibitor and CMV status of the recipient in HTX recipients. (p=1,000).

		status of the recipient		
		CMV negative	CMV positive	All
CMV infection after mTOR	quantity	0	2	2
	of CMV infection (%)	0	100	100
	of particular CMV status of the recipient (%)	0	7,7	5,4
No CMV infection after mTOR	quantity	11	24	35
	of particular CMV status of the recipient (%)	100	92,3	94,6

Table 11: CMV infection in heart transplant recipients after start with mTOR inhibitors related to the CMV status of the recipient

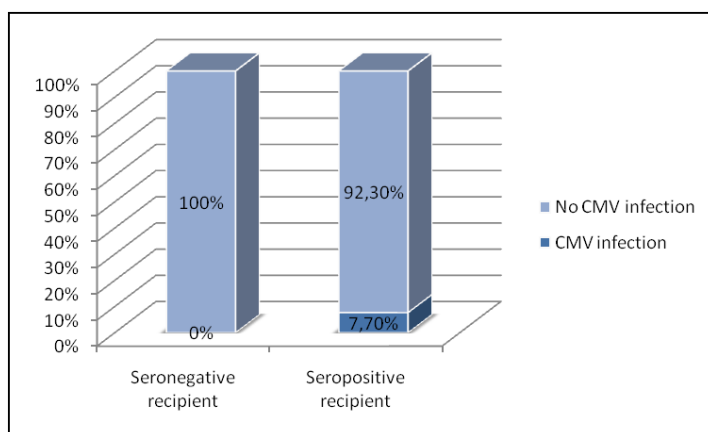


Figure 14: CMV infection in HTX recipients related to the CMV status of the recipient

CMV infection related to the kind of mTOR inhibitor

When looking at HTX patients who did not develop CMV infection after mTOR inhibitor medication:

- 31 of them had Everolimus in their immunosuppressive regimen correlating to 81,6% of HTX patients where CMV infection did not occur, and 93,9% of those who received Everolimus.
- 7 were immunosuppressed by Sirolimus, what is equivalent with 18,4% of HTX patients who did not develop CMV infection, and 100% of Sirolimus treated HTX recipients.

Considering the HTX patients infected by CMV after they received a mTOR inhibitor as a part of their immunosuppression:

- 2 of them received Everolimus, making 100% of the CMV infected patients who underwent HTX, and 6,1% of those HTX recipients who were medicated with Everolimus.
- None of the CMV infected HTX recipients had Sirolimus as a therapy.

No significance between the occurrence of CMV infection after using a mTOR inhibitor as part of immunosuppression and the kind of used mTOR inhibitor could be attested. ($p=1,000$)

		Everolimus	Sirolimus	All
CMV infection after mTOR	quantity	2	0	2
	of obtained mTOR inhibitor (%)	100	0	100
	of CMV infection (%)	6,1	0	5
No CMV infection after mTOR	quantity	31	7	38
	of obtained mTOR inhibitor (%)	93,9	100	95

Table 12: CMV infection in heart transplant recipients related to the kind of mTOR inhibitor they obtained

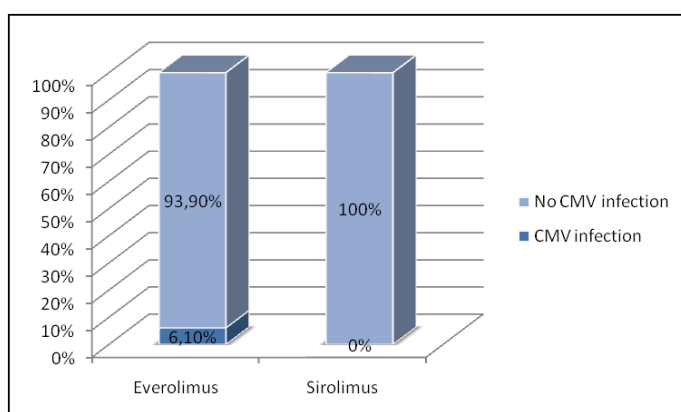


Figure 15: CMV infection in HTX recipients related to the mTOR inhibitor the received

4.3 Liver transplantation

CMV infection related to the CMV status of the donor

In those LTX recipients who did not develop CMV infection there were 20 cases whose donor had a negative CMV status correspondent with 60,6% of the LTX patients who got their organ from a CMV negative donor, and 43,5% of those LTX recipients who did not develop CMV infection. In 26 LTX recipients where CMV infection did not occur, the donor was CMV positive, that are 78,8% of patients who underwent LTX, getting the organ from a donor with CMV negative status, and 56,5% of those LTX recipients who did not develop CMV infection.

From patients who underwent LTX there were 13 who developed CMV infection and got their liver from a donor with a negative CMV status, which makes 39,4% of LTX recipients getting an organ from a CMV negative donor, and 65% of LTX patients who got infected by CMV. In LTX recipients who contracted a CMV infection, 7 got an organ from a CMV positive donor, corresponding with 21,2% of LTX recipients getting an organ from a positive donor, and 35% of LTX patients who developed CMV infection.

No significant relation between CMV infection after immunosuppression with mTOR inhibitors and the CMV status of the donor could be shown in liver transplant recipients. ($p=0,180$).

		status of the donor		
		CMV negative	CMV positive	All
CMV infection after mTOR	quantity	13	7	20
	of CMV infection (%)	65	35	100
	of particular CMV status of the donor (%)	39,4	21,2	30,3
No CMV infection after mTOR	quantity	20	26	46
	of particular CMV status of the donor (%)	60,6	78,8	69,7

Table 13: CMV infection of liver transplant recipients after start with mTOR inhibitors related to the CMV status of the donor

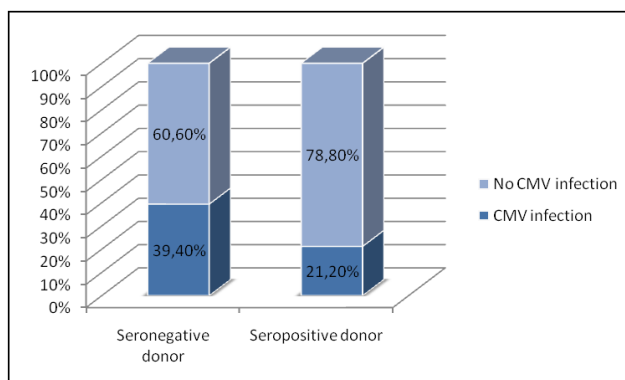


Figure 16: CMV infection in LTX recipients related to the CMV status of the donor

CMV infection related to the CMV status of the recipient

From those LTX recipients, who developed CMV infection there were 7 cases of negative CMV status, coinciding with 36,8% from LTX patients with negative CMV status, and 41,2% of those who got infected by CMV. 10 cases got CMV infection and had a positive CMV status, what is equivalent with 27% of the LTX recipients with positive CMV status, and 58,8% of those who developed CMV infection.

In 12 LTX cases where no CMV infection occurred the CMV status of the recipient was negative, meaning 63,2% of LTX patients with a negative CMV status, and 30,8% of those who did not develop CMV infection. 27 LTX recipients had a positive CMV status and did not get infected by CMV after mTOR inhibition, correspondent with 73% of positive CMV status, and 69,2% of the LTX patients not infected by CMV.

Our study could not find a significance between CMV infection after a mTOR inhibitor was prescribed and CMV status of the recipient in LTX patients. (p=0,543).

		status of the recipient		
		CMV negative	CMV positive	All
CMV infection after mTOR	quantity	7	10	17
	of CMV infection (%)	41,2	58,8	100
	of particular CMV status of the recipient (%)	36,8	27	30,4
No CMV infection after mTOR	quantity	12	27	39
	of particular CMV status of the recipient (%)	63,2	73	69,6

Table 14: CMV infection of liver transplant recipients after start with mTOR inhibitors related to their CMV status

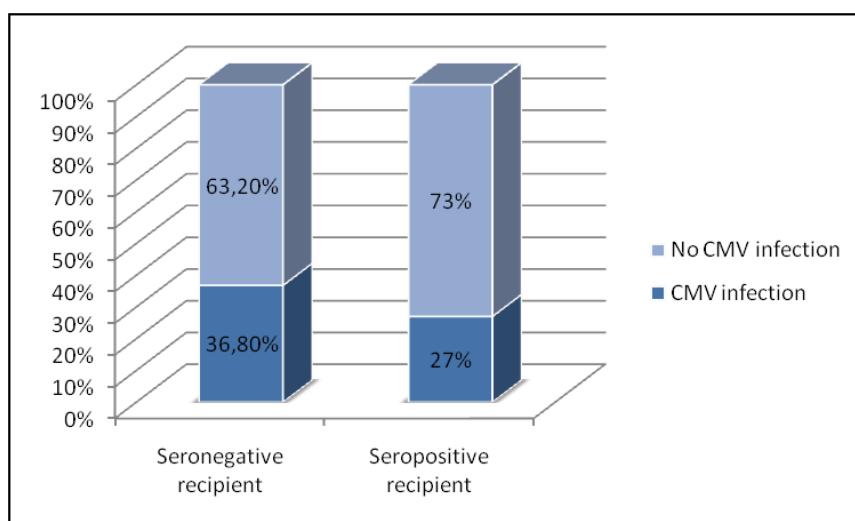


Figure 17: CMV infection in LTX recipients related to the CMV status of the donor

CMV infection and kind of immunosuppression

Considering the LTX recipients who developed CMV infection after immunosuppression with mTOR inhibitors:

- None of them had Everolimus in their immunosuppressive regimen.
- 20 received Sirolimus as a part of their immunosuppression, what are 32,3% of the Sirolimus treated LTX patients, and 100% of CMV infections in patients who underwent LTX.

Regarding those LTX recipients who did not develop CMV infection after they received a mTOR inhibitor as a part of their immunosuppression:

- 7 of them received Everolimus, which coincides with 100% of Everolimus treated LTX patients, and 14,3% of those who did not develop CMV infection.
- 42 cases had Sirolimus on the list, making 67,7% of Sirolimus treated patients who underwent LTX, and 85,7% of CMV infection in LTX patients.

There could not be shown any significant relation in LTX recipients between CMV infection after mTOR inhibition and the kind of mTOR inhibitor they received for immunosuppression. ($p=0,098$).

		Everolimus	Sirolimus	All
CMV infection after mTOR	quantity	0	20	20
	of obtained mTOR inhibitor (%)	0	32,3	29
	of CMV infection (%)	0	100	100
No CMV infection after mTOR	quantity	7	42	49
	of obtained mTOR inhibitor (%)	100	67,7	71

Table 15: CMV infection of liver transplant recipients after start with mTOR inhibitors related to which mTOR inhibitor they obtained

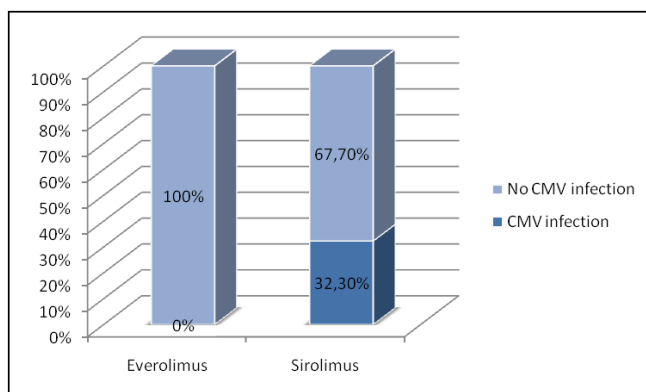


Figure 18: CMV infection in LTX recipients related to the mTOR inhibitor received

4.4 CMV – mismatch

CMV mismatch related to CMV infection

6 cases of CMV mismatched recipients developed CMV infection, equivalent with 31,6% of those who got infected by CMV, and 15,4% of those with positive CMV mismatch. In 33 organ recipients where the CMV mismatch was positive, CMV infection did not occur, making 45,8% of those patients who did not develop CMV infection, and 84,6% of positive CMV mismatch.

When the CMV mismatch was negative 39 transplanted patients did not develop CMV infection after immunosuppression with a mTOR inhibitor, that makes 54,2% of those who did not get infected by CMV, and 75% of those who had a negative CMV mismatch. 13 cases of negative CMV mismatch developed CMV infection, equivalent with 68,4% of those who got infected by CMV, and 25% of negative CMV mismatch.

We could not show a significant relation between the CMV mismatch and the occurrence of CMV infection after therapy with mTOR inhibitors. ($p=0,307$)

		CMV mismatch	No CMV mismatch
CMV infection after mTOR	quantity	6	13
	of CMV infection (%)	31,6	68,4
	of CMV mismatch state (%)	15,4	25
No CMV infection after mTOR	quantity	33	39
	of CMV mismatch state (%)	84,6	75

Table 16: CMV mismatch related to CMV infection after start with mTOR inhibitors

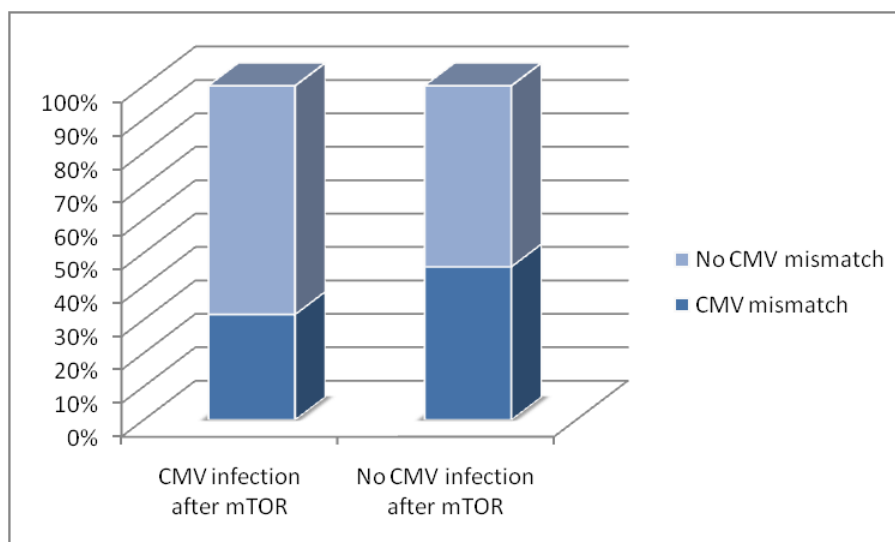


Figure 19: CMV mismatch related to CMV infection after start with mTOR inhibitors

5 Discussion

Transplantation of the liver is still the only option to treat patients with end stage liver disease, like cirrhosis due to several underlying diseases (1), cholestatic disease, primary liver tumours, acute hepatic failure and various other diseases (4). The technique of first choice in the Division of Transplantation Surgery of the Medical University Graz, is the “Piggyback” technique, a modified version of the conventional one where no venous-venous bypass is needed (18).

Cardiac transplantation is the therapy of choice in patients suffering from end-stage heart failure, where all other treatment options have failed (12). The operative technique has not changed very much since its first description from Lower and Shumway in 1967 (21) (22).

There are lots of complications in both, LTX and HTX, from primary non-function (28), acute and chronic rejection (24) (28), to side effects of immunosuppressants (30) (33), malignancies (28) (30) and many other more. Moreover infections are a serious complication in transplantation, and here CMV infection is one of the most important (26) (30).

The CMV is a DNA-virus belonging to the group of herpesviridae (35). It is transmitted in different ways, also in organ transplantation (37). So transplant recipients are at high risk to develop CMV infection (39). CMV infection can have two different effects. Either direct effects (as CMV syndrome or as tissue invasive disease) (38), or indirect effects (41).

For diagnostic purposes there are different methods available: serology, used to identify the CMV status of donor and recipient (39); viral cultures (36); antigenemia assays, being fast and highly specific for CMV by identifying pp65, a lower-matrix protein of CMV (42); nucleic acid amplification, detecting DNA or RNA of the virus by PCR (43); or nucleic acid hybridization (42).

The prevention of CMV infection is an important procedure in the management of transplant recipients. There are two main strategies in prevention: the preemptive therapy, where CMV is monitored aggressively and therapy is started before it comes to symptomatic disease (39); the second strategy is antiviral prophylaxis, where antiviral medication is administered to patients who are at risk to develop CMV infection (44).

Active CMV infection after solid organ transplantation can be treated either with IV ganciclovir or oral valganciclovir. Moreover it is important to reduce immunosuppression. Treatment of CMV infection should be individualized, and monitored by surveillance of CMV persistence (39).

A relatively new class of immunosuppressants are the so called mTOR inhibitors, represented by Sirolimus and its derivate Everolimus (50). This new substance class is, amongst others, assumed to reduce the risk of developing CMV infection in solid organ transplant recipients (67). First Sirolimus was found in a fungus growing in the soil of Rapa Nui (51), and before its immunosuppressive activity was discovered, its potent antifungal and antitumor effects were detected (50).

mTOR inhibitors interact with FKBP (a family of intracellular binding proteins) and build an immunocomplex (51). The target of this complex is the so called mammalian target of rapamycin (mTOR) (53) located at the branchpoint of the signal 3 pathway in activated T-cells (54). By inhibiting mTOR, G1 to S transition, translation and cytokine-driven T-cell proliferation is inhibited (50). mTOR inhibitors also block growth factor driven proliferation of hematopoietic and non-hematopoietic cells, including bronchial and vascular smooth muscle cells (55). Furthermore Sirolimus and Everolimus inhibit B-cell activation, proliferation and differentiation into antibody producing cells (50).

Besides the immunosuppressive effects, mTOR inhibitors have antifungal, antitumor properties and prevent from graft vascular disease (75). Low rejection rates in transplant recipients medicated with mTOR inhibitors have been shown before, when combined with Cyclosporine even lower rejection rates (57; 59). Other positive aspects reported, are the absence of common side effects other immunosuppressants have, like nephrotoxicity, neurotoxicity, diabetes mellitus and hypertension (50). But mTOR inhibitors also have adverse effects. The most common are hyperlipidemia, lung problems, proteinuria, pancytopenia, lymphocele, wound infection and impaired wound healing (53). Furthermore hematomas, hernias, arthralgia, elevated liver enzymes, diarrhea, fatigue and nausea (64).

It has been claimed before, that mTOR inhibitors show a lower CMV infection rate in solid organ transplant recipients (76).

Our question was, how Sirolimus respectively Everolimus affect the development of CMV infection, and if there is a difference between these two mTOR inhibitors. Moreover we wanted to know if there is a difference in the appearance of CMV infection between liver and heart transplant recipients. Another point was to evaluate the occurrence of CMV infection regarding the CMV status of the donor or rather the status of the recipient.

CMV infection related to the transplanted organ:

In our study we found out that in liver transplant recipients significantly more patients developed CMV infection than in heart transplant recipients (29% in liver transplant recipients vs. 5% in heart transplant recipients). And altogether 20,2% of transplant recipients developed CMV infection.

One study from Spain, not correlating with ours, showed an occurrence of CMV infection in heart transplant recipients of 54,05% (20 out of 37 recipients), defined by pp65 positive blood samples (77). Others reported that in 69 heart transplant recipients 11,5% got infected by the CMV (78). Sarmiento et al. found out that 21% (8 out of 38) of cardiac transplant recipients developed CMV infection after transplantation. They proved CMV infection by shell vial assay or antigenemia (pp65) (79) Another study reported that they detected 10,1% (11 out of 103) heart transplant recipients developing PCR positive CMV infection (80). Mattila et al. noted in their study, that in their 190 heart transplant recipients from the year 1985 to 1995, 29 patients developed CMV infection, which makes 15,2% (81).

In liver transplant recipients Hoppe et al. suggested that 65,8% of them got infected by CMV, detected by antigenemia (pp65) (82). One study by Lautenschlager et al. showed an incidence of CMV infection after LTX of 11% (26 out of 229 patients). The infection was confirmed by pp65-antigenemia (83). Other authors demonstrated in a previous study that 44% (74 recipients out of 169) of their liver transplant recipients showed CMV infections discovered by positive pp65-antigenemia assays (84). The experience of Oldakowska-Jedynak et al. pointed out that in their department 30% (37 liver transplant recipients out of 123) developed CMV infection after LTX, measured by antibody serology (85).

Because there are no studies comparing the difference of developing CMV infection in HTX and LTX recipients, but only looking at the CMV infection either in HTX or LTX recipients, it is not possible to show whether our findings, that in LTX recipients CMV infection occurs significantly more often than in HTX recipients,

coincides with other results. Moreover, data found in the literature for the incidence of CMV infection in liver transplant recipients or in cardiac transplant recipients, is widely spread.

All transplant recipients included in our study received a mTOR inhibitor, either Sirolimus or Everolimus, and the overall CMV infection rate was 20,2%. Comparing it with studies where no mTOR inhibitor was used, the infection rate ranged widely from 10,1% (80) to 65,8% (82). So ours is somewhere between and we could not say that in transplant recipients, where mTOR inhibitors is a part of their immunosuppression, the incidence of CMV infection is lower. But it is difficult to compare these studies due to the diverse study designs they show. Furthermore our study population is not as big as some listed in other studies, which is another difficulty in comparing.

CMV infection related to the kind of mTOR inhibitor

One outcome of our study was that transplant recipients developed significantly more CMV infections when they were immunosuppressed by Sirolimus (29%) as when they received Everolimus (5%) in their immunosuppressive regimen.

Papanikolaou V et al. claimed in their study that they found out that their Everolimus treated kidney recipients showed an incidence of 8,33% in development of CMV infection (86). Another study suggested for the occurrence of overall CMV infection in heart transplant recipients 7,7% for patients with an Everolimus dose of 1,5mg per day, 7,6% for those with an Everolimus dose of 3mg per day and 21,5% for those who were medicated with Azathioprine (87). One publication described an incidence of CMV infection in renal transplant recipients of 2,6% (3 recipients out of 113) when treated with Everolimus either with or without oral steroids (88). Haverich et al. cited in their international, double-blind trial a CMV infection rate of 8,6% (for Everolimus dose of 1,5mg per day) and 8,1% (for Everolimus dose of 3mg per day) in de novo heart transplant recipients (89).

The information found in the literature about Everolimus and its outcome on CMV infection coincides approximately with our findings.

Trotter et al. reported the almost absence of CMV infection (<2%) in their group's cohort of Sirolimus patients, composed of liver transplant recipients (57). Others have demonstrated that liver transplant recipients receiving a combination of Sirolimus and Tacrolimus showed an appearance of CMV infection in 7% of their

cases (4 out of 56). They recommended a combination of Sirolimus and Tacrolimus in liver transplant recipients because of their synergistic effect (90). One study which included 83 kidney transplant recipients from 11 centres from Europe, found out that those who were prescribed Sirolimus as part of their immunosuppression got infected by CMV in 14% of the organ recipients (91). In a large study from Kahan, where 1008 kidney transplant recipients took part, only 7% (37 recipients) of all showed infection with the CMV (92).

These results showed far fewer CMV infections in Sirolimus treated patients than our study did.

There were no studies found comparing Sirolimus and Everolimus, looking at the difference in appearance of CMV infection. There were only studies found, where the incidence of CMV infection in patients receiving either Sirolimus or Everolimus was listed.

Therefore we could not show if our finding that Sirolimus treated transplant recipients develop significantly more CMV infection than Everolimus treated do, correlates with studies done before. The incidence of CMV infection in patients with Everolimus as part of their immunosuppression of 5% is similar to other data, which ranges from 2,6% (88) and 8,6% (89) and is listed above. Contrary, the Sirolimus group in our study showed a higher CMV infection rate (29%) than other studies report ranging from less than 2% (57) to 14% (91) as mentioned before. But the studies are difficult to compare with, because the immunosuppression is not standardized. Moreover, some use a defined dose of immunosuppression, and in some, like in our transplant recipients, the dose of immunosuppression is adapted by controlling the level of immunosuppression in blood samples.

CMV infection related to the CMV status of the donor

We have observed that there is no significant difference in developing CMV infection in our study population whether they received their organ from a seronegative donor or from a seropositive donor. From those patients who got infected by CMV after start with mTOR inhibitors, 59,1% had a seronegative donor and 40,9% had a seropositive donor. In those who got their organ from a seronegative donor 25% developed CMV infection, and in those who got their organ from a seropositive donor 17,3% got infected by the CMV.

Hughes et al. found in their study, regarding 486 renal transplant recipients, that the impact of donor serostatus on CMV antigenemia was substantial. Organs from

seropositive donors were associated with 49% infection rate in the recipients, compared to a 19% infection rate when the donor was seronegative. They also used the pp65 antigenemia assay to prove CMV infection (93)

Maybe there is a difference in kidney transplant recipients to heart and liver transplant recipients in the overall occurrence of CMV infection. Moreover all of their patients received triple therapy with cyclosporin A, methylprednisolone and azathioprine (93), and no mTOR inhibitor. Our transplant recipients showed no significant difference, but the overall infection rate was lower than in the study listed above.

Another study with liver transplant recipients detected also that in significantly more transplant recipients with seropositive donors CMV infection occurred than in those who had seronegative donors. Seropositive donors were linked with an infection rate in the recipients of 23,1% (56 recipients out of 242), whereas recipients with seronegative donors showed an infection rate of only 9,7% (23 patients out of 236). They also did not have a mTOR inhibitor in their immunosuppressive regimen (94). Although the transplant recipients in this study did not received mTOR inhibitors, the overall infection was not higher than in our transplant recipients.

One study, supporting our results, reported that CMV status of the donor did not reach statistical significance as a risk factor for CMV infection in their LTX recipients. These patients also did not get mTOR inhibitors as immunosuppression (95)

CMV infection related to the CMV status of the recipient

We found out that there is no significant relation between development of CMV infection and the CMV status of the recipient. In 23,3% of seronegative patients CMV infection occurred, compared with 19% in seropositive patients.

Neumann et al. could not affirm this result. They found a significant higher incidence of CMV infection in liver allograft recipients whose CMV status was negative prior to transplantation than those whose CMV status was positive (94). Another study arrives the same conclusion like we did. They reported that there is no significant difference in the CMV infection rate depending on the CMV status of the recipient in their kidney transplant recipients: 35% in seronegative recipients and 36% in seropositive recipients (93).

CMV infection related to the mTOR inhibitor the recipients received

In our study significantly more HTX recipients received Everolimus as part of their immunosuppressive therapy Than LTX recipients did. And significantly more LTX patients received Sirolimus than HTX recipients did.

No previous study could be found to show if this result correlates with others. But there are many reports approving the benefit effects of Everolimus (67; 70; 71; 96; 74) or Sirolimus (97; 98; 99; 100) in HTX recipients.

Various surveys have been made, attesting the benefit use of Everolimus (101; 102; 103; 104) or Sirolimus (105; 53; 106; 107) in LTX recipients.

CMV mismatch related to the occurrence of CMV infection

We could not find a significant relation between the CMV mismatch of donor and recipient related to appearance of CMV infection in the organ recipients. In CMV mismatched recipients CMV infection occurred in 15,4%, in not mismatched recipients CMV infection developed in 25% of the cases.

Hughes et al. could not prove our finding. In their renal transplant recipients CMV infection occurred significantly more often in mismatched patients than in not mismatched ones (41,8% in mismatched vs. 29,14% in not mismatched) (93). Another study from the Netherlands also came to the same result, that in CMV mismatched transplant recipients CMV infection occurred more often (108). Neumann et al. shared the outcome of our study. In their LTX recipients no significant relation between CMV mismatch and occurrence of CMV infection could be attested, showing a CMV infection rate of 16,2% for not mismatched recipients and 16,8% for mismatched patients (94).

Conclusion

Our results are difficult to compare with previous studies because the immunosuppressive regimen differs in each study and, what is more, no standardized immunosuppressive regimen was used in our study, but an immunosuppressive therapy that is adjusted on the blood level of the immunosuppressive drugs. Moreover the study designs differ from study to study. Another fact is that the group of our recipients is quite small, therefore no definite statements can be given. Besides, we did not have a comparison group to the mTOR inhibitors Sirolimus and Everolimus. Another problem is that there is a possible bias in our study due to the fact that most of the LTX patients received Sirolimus and most of the HTX recipients received Everolimus. For this reason our

finding that CMV infection occurs significantly more often in Sirolimus treated patients could be in consequence of our result that significantly more liver transplant recipients developed CMV infection, or vice versa. Therefore further investigations have to be performed.

6 References

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