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ORIGINAL ARTICLE

Multicentric outcome analysis of sirolimus-based immunosuppression in 252 liver transplant recipients

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Summary

The use of sirolimus (SRL) in orthotopic liver transplantation (OLT) has been controversial after experimental data suggested an increased risk of hepatic artery thrombosis (HAT). To assess the safety and efficacy of SRL as de novo immunosuppression in OLT recipients. Outcomes of 252 OLT patients who received SRL were compared with outcomes of 291 OLT recipients who received calcineurin inhibitor in a retrospective study. Primary outcomes of this study were: patient- and graft survivals, vascular, biliary, wound complications and rejection rates. Secondary outcomes were: postoperative infection rate, bone marrow and renal function and changes of lipid levels. Patient- and graft survivals, rejection and infection rates were similar. In the SRL group, HAT occurred in 1.2%, biliary complications in 19.4%, and incisional hernias in 9.1%. In the control group the incidence of HAT was 5.8% ($P = 0.004$), biliary complications 18.5% (P = NS) and incisional hernias 7.2% (P = NS). Patients on SRL experienced significantly higher levels of serum triglycerides but fewer acute cellular rejections. Bone marrow and renal functions were similar in both the groups. Our findings would suggest that SRL is safe and effective for very selected OLT recipients. Randomized controlled trials are necessary to confirm our results.

Introduction

Since the introduction of Cyclosporine (CyA) [1], patients undergoing orthotopic liver transplantation (OLT) have achieved 5-year survival rates between 70 and 85% [2] and a long life-expectancy with consequent risk of developing long-term side-effects of immunosuppression medications [3–5]. The introduction of more recent compounds such as tacrolimus (TAC), mycophenolate mofetil (MMF), and sirolimus (SRL), has allowed physicians to choose from a larger number of medications and tailor the immunosuppression drugs to minimize their side-effects. Nevertheless, none of the immunosuppressive protocols has shown clear advantages over others [6,7]. SRL has no effect on calcineurin, and therefore it is an attractive drug for patients with renal impairment or for individuals who are intolerant to calcineurin inhibitors (CNIs) [8–10]. Despite the initial optimism, the preliminary results of a multicentric phase II trial showed an increased incidence of hepatic artery thrombosis (HAT), graft loss and inferior survival rate in the SRL arm that led the Food and Drug Administration (FDA) to place a black box label on SRL for OLT recipients [6,11–13]. Consequently, experience with SRL in OLT has been very limited and there is a modest body of literature on its effectiveness and safety in this setting. The aim of this study was to analyse the outcomes of a large cohort of patients ($n = 252$) who received SRL as the primary immunosuppressant medication in two university hospitals. Primary and secondary outcomes were

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compared with a cohort of OLT recipients who received an OLT and were treated with CNI during a similar time interval.

Patients and methods

Study design

A retrospective study on the effectiveness and safety of the use of SRL in adult OLT recipients was performed by comparing two cohorts of patients undergoing OLT at the University of Alberta (December 1996–December 2003) and at the University of Colorado (February 1998 and May 2002). One cohort (n = 252) received de novo SRL and another cohort (n = 291) received CNI immunosuppression during the same period (Fig. 1). Ethics review committees of the two participating centers approved this study. At the University of Alberta, written informed consent was obtained from each participant receiving SRL after the issuance by FDA of a 'black box' warning and at both centers, no patients refused SRL therapy when recommended by their caregivers.

Primary and secondary outcomes of this study

Primary outcomes of this study were the following: (i) 5-year patient survival rate, (ii) 5-year graft survival rate,

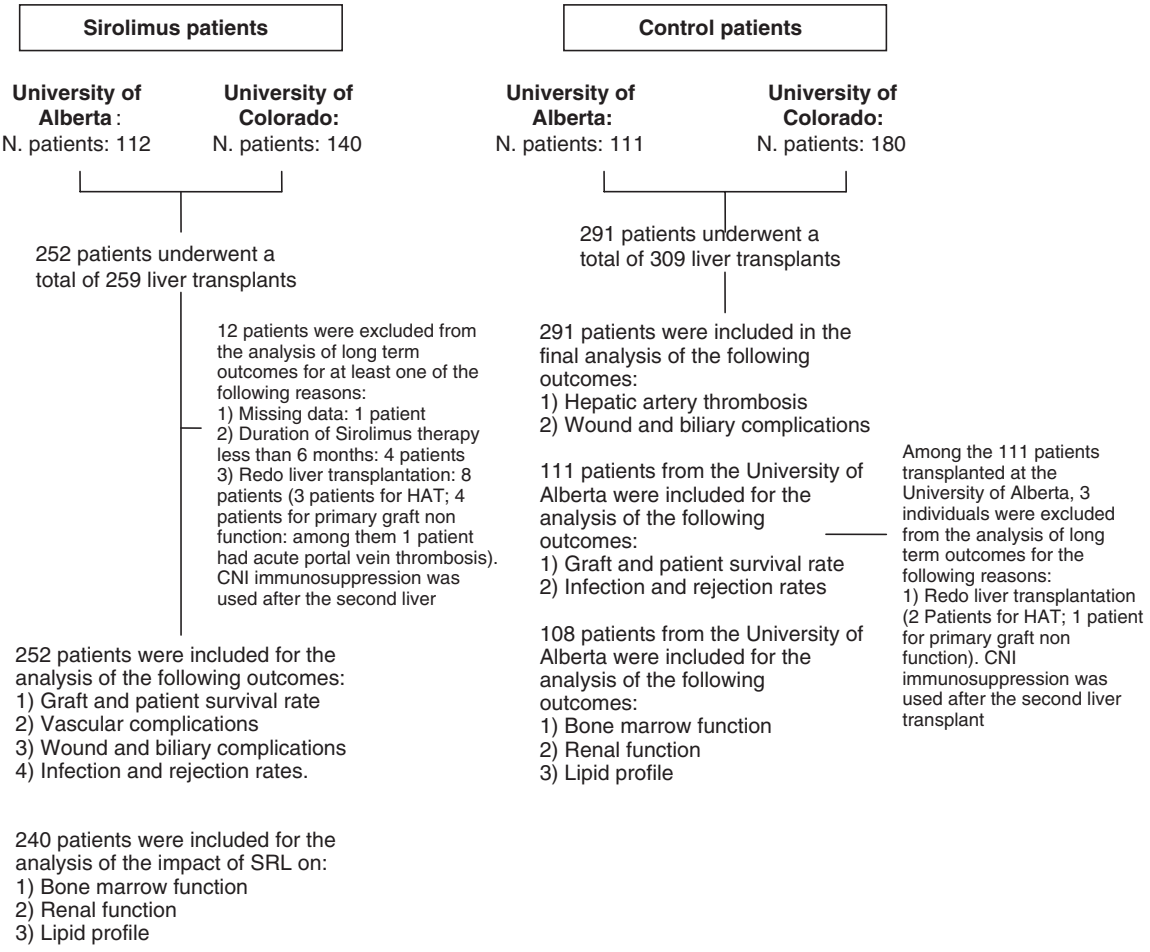

Figure 1 Flow diagram of the study population treated with sirolimus and the control group. From December 1996 until December 2003, a total of 252 patients underwent 259 cadaveric liver transplants at the University of Alberta and at the University of Colorado medical centers with sirolimus therapy used as primary immunosuppression. Twelve patients were excluded from the final analysis of long-term complications of sirolimus because of the duration of sirolimus therapy was less than 6 months, because of retransplantation or missing data. The control group consisted of 291 patients who underwent 294 cadaveric liver transplants at the University of Alberta from January 1997 until December 2002 and at the University of Colorado from January 1997 until December 1999. Among the control group, patient- and graft survival analysis and evaluation of long-term outcomes were possible only for the cohort of patients transplanted at the University of Alberta as data from the University of Colorado were not available.

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(iii) graft rejection rates, (iv) incidence of vascular thrombotic events, (v) incidence of biliary complications and (vi) incidence of wound complications.

Similarly, statistical analysis was carried out to assess any differences for the following secondary endpoints: (i) the rate of postoperative infections, (ii) long-term bone marrow function, (iii) long-term renal function measured by variation of serum creatinine, and (iv) serum lipid levels.

Sirolimus-based immunosuppression protocol

All 252 adult patients received SRL during the first 24 h after surgery. At the University of Alberta, 112 individuals received SRL in addition to reduced dosages of CNI (TAC or CyA). The administration of intravenous steroid (Solumedrol 500 mg) was used for induction immunosuppression and substituted by interleukin 2 receptor antagonists (daclizumab 1 mg/kg IV on days 0 and 5) during the last 3 years of this study. The oral dose of SRL was adjusted to keep the blood levels in the range of 10–15 ng/ml during the first 3–6 months and then in the range of 5–10 ng afterwards. CNI and steroids immunosuppressive regimen were tapered off completely by the end of 3–6 months.

At the University of Colorado, a total of 140 consecutive patients undergoing OLT received SRL (6 mg on day 0 followed by 2 mg/day) in addition to a 3-day course of corticosteroid and CNI. Among them, three (2.1%) patients received a graft from a living donor.

Primary indications for the use of sirolimus

At Denver, SRL-based immunosuppression was mainly used for recipients with renal dysfunction or intolerance to CNI. At the University of Alberta, SRL was prescribed routinely for patients with hepatocellular carcinoma (HCC) and for a cohort of 57 consecutive adults transplanted between July 2000 and October 2001 who were enrolled in a pilot study to evaluate the effectiveness and safety of de novo SRL therapy.

Inclusion and exclusion criteria for patients on sirolimus

The inclusion criteria for patients on SRL were the following: adult age, SRL therapy started in the first 24 h after undergoing OLT, SRL therapy continued for at least six continuous months.

On the other hand, patients under the age of 18, individuals who were enrolled in clinical trials that did not allow the use of SRL and recipients of living donor grafts (only at the University of Alberta Hospital) were excluded from this study. In addition, 12 patients were excluded from the analysis of long-term outcomes for at least one of the following other reasons:

1 missing data: one patient

2 duration of sirolimus therapy less than 6 months: four patients

3 redo liver transplantation: eight patients (three patients for HAT; four patients for primary graft nonfunction: among them one patient had acute portal vein thrombosis). CNI immunosuppression was used after all these patients underwent a second OLT.

CNI-based immunosuppression protocol

At both transplant centers, a total of 291 individuals were enrolled in the control group and received oral CNI immunosuppression.

At the University of Alberta, 111 patients received CNI immunosuppression: 13 (11.7%) patients who were transplanted during the period between 1996 and 1999 received CyA (blood trough level kept in the range of 150–200 ng/ml) while the remaining 99 patients (89.1%) were transplanted between 2000 and 2003 and received TAC (blood trough level kept in the range of 3–6 ng/ml). Induction was effected by intravenous steroids (Solumedrol, 500 mg), with rapid postoperative tapering to 20 mg/day of prednisone by day 5 and complete discontinuation by month 3 as tolerated.

At the University of Colorado, a total of 180 patients received CNI-based immunosuppression. During the period between 1998 and 2002, CyA and TAC were used with equal frequency according to individual clinician's preference. After 2002, CyA was discontinued altogether as a change in practice at that center. As a consequence, 79 (43.8%) individuals were treated with CyA (blood trough level kept in the range of 200–250 ng for month 1, 175–200 ng for month 2 and 150 ng thereafter) and 101 (56.1%) with TAC (blood trough level kept in the range of 10–15 ng/ml for month 1, 8–12 ng/ml for month 2 and 5–8 ng/ml thereafter) plus 14-day corticosteroid taper with or without mycophenolate mofetil [14]. Among them, two participants (1.1%) received grafts from living donors.

Inclusion and exclusion criteria for patients in the control group

The inclusion criteria for patients on CNI were the following: adult age, CNI therapy started in the first 24 h after undergoing OLT, CNI therapy continued for at least six continuous months.

On the other hand, patients under the age of 18 were excluded from this study. In addition, among the 111 patients transplanted at the University of Alberta, three individuals were excluded from the analysis of long-term

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outcomes as they underwent a second OLT: two patients for HAT and one patient for primary graft nonfunction.

Supplemental drug therapies used for both cohorts during the study period

At both transplant centers, supplemental drugs were similarly used to prevent well-known infections and cardiovascular complications typical for immunosuppressed patients. Oral Trimethoprim-sulfamethoxazole was administered (80 mg/400 mg three times a week) up to 3 months as prophylaxis for *Pneumocystis carinii* infection [15]. Prophylaxis with oral ganciclovir was continued up to 6 weeks for patients who were cytomegalovirus (CMV) IgG negative prior to transplant and who received an organ from a CMV IgG positive donor [16]. The use of antihypertensive medications, lipid-lowering drugs, insulin and oral hypoglycemic agents, among all the other medications, were prescribed according to established guidelines by the American Heart Association (AHA) and American College of Cardiology (ACC) [17].

Data collection

Two independent investigators extracted data from digital or paper files and entered in a computerized database. Less than 5% of data was missing. MELD scores were calculated retrospectively for each patient at the time of transplantation as this study enrolled the majority of patients prior to the use of MELD for organ allocation [18]. After cross analysis of the data, all the discrepancies were verified from the original documents and random quality of data entry was performed until no further rectifications were necessary.

Data collected during the first 12 months after OLT

Perioperative wound complications and infections were classified as early (<6 months) and late (>6 months) after OLT. Body fluid cultures were obtained on clinical suspicion and no protocol cultures were performed in asymptomatic recipients.

Data collected during the 5-year period after OLT

Vascular complications, biliary ducts anastomotic complications, incisional hernias, rejections, graft and patient survival rate were collected for the entire 5-year period of follow-up. Blood trough levels of SRL [19], TAC, and CyA, transaminases (AST, ALT, Total Bilirubin, ALP, LDH), serum creatinine, bone marrow function tests (white blood cell count (WBC), platelet count and hematocrit) and lipid profiles (serum cholestertol and triglycerides levels) were analysed at 1 month, 6 months, 12 months and then yearly until 5 years after OLT.

Definitions

Outcome definitions of this study have been previously reported in the literature as they are frequent events in critically ill patients:

Blood infections: Presence of two positive distinctive cultures or a positive culture from the tip of central venous catheters associated with one positive blood culture [20].

Urinary tract infections: Presence of more than 10^5 colony forming units (CFU) in the urine samples of symptomatic patients [21].

Pulmonary infections: Presence of at least two of the following findings: (i) positive sputum culture, (ii) gram stain with dominant organism and presence of many polymorphonuclear leukocytes in the sample obtained by suctioning of the respiratory tract or by sputum, or (iii) presence of radiologic infiltrate on chest radiograms or CT scans [22].

Cytomegalovirus infections (CMV): Presence of CMV immunoglobulin M antibodies in the blood or viral inclusion bodies on liver biopsy specimens. CMV-DNA levels were measured in all such patients to confirm the diagnosis. Immunohistochemical staining was performed on suspicious biopsy specimens [23].

Vascular complications: Presence of at least one of the following findings diagnosed by imaging tests or during exploratory laparotomy: (i) hepatic artery thrombosis, (ii) portal vein thrombosis, (iii) critical stenosis of hepatic artery resulting in graft ischemia, (iv) hepatic artery pseudo-aneurysm or rupture or (v) any combination of the above conditions [24].

Biliary complications: Presence of at least one of the following findings diagnosed by imaging tests or during exploratory laparotomy: (i) biliary duct stricture at the anastomotic site or in any of the intra- and extra-hepatic bile ducts, (ii) bile extravasation resulting from an interruption of continuity of the intra- or extra-hepatic bile ducts, (iii) circumscribed collection of bile in the peritoneal cavity (biloma), (iv) intra-abdominal abscess caused by superinfection of bile collecting outside the biliary tree and (v) any combination of the above conditions [25].

Wound complications: Wound complications were defined by any of the following: (i) superficial abscess or fluid collection requiring drainage by opening of the incision, (ii) presence of infected tissue necrosis along the incision, (iii) skin or subcutaneous tissue dehiscence along the incision in the presence of positive cultures or gram stains obtained from the incision or (iv), skin and

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subcutaneous tissue infection with fascial dehiscence [26].

Graft rejection: Rejections were diagnosed by liver biopsy or by liver functions tests elevation (two or more times the normal serum range) in the absence of other causes graft dysfunction with normalization of their values after at least one of the following interventions:

1 increased dose of established immunosuppression regimen,
2 pulse steroid treatment or
3 anti-leukocyte antibody infusion therapy.

Investigations used for the diagnosis of vascular and biliary complications

Suspected vascular or biliary complications (most commonly evidenced by rising liver function tests) were evaluated by Doppler ultrasound of the liver graft as a first imaging test when clinically indicated; no protocol imaging studies were performed at either transplant center for the investigation of asymptomatic patients for potential biliary or vascular adverse events. Triphasic contrast computerized tomography (CT scan) or magnetic resonance imaging/magnetic resonance cholangio-pancreatography (MRI/MRCP), angiography, nuclear scintigraphy scans/hydroxyiminodiacetic acid (HIDA) scan, and endoscopic retrograde cholangiography (ERC) or percutaneous transhepatic cholangiography (PTC) were obtained whenever appropriate if the Doppler ultrasound test was abnormal.

Statistical analysis

Student's *t*-test was used for statistical analysis of continuous variables and analysis was performed by chi-squared test for binary data or Fisher's exact test when appropriate. Kaplan–Meier technique was used for survival analysis and the log-rank test was used to assess differences between the two groups. All *P* values were two-sided and considered statistically significant when less than 0.05.

Results

Subjects

Demographic and clinical characteristics were comparable between the two groups except for the median follow-up that was longer for recipients treated with CNI immunosupression as SRL had been used more frequently in the later period of this study (3.9 years vs. 7.1 years; $P = 0.001$) and for the fact that HCC was more frequently observed in the group of patients on SRL (10.4% vs. 0.3%; $P = 0.003$). On the other hand, autoimmune disease was more prevalent in the cohort of patients on CNI immunosuppression (8.5% vs. 0%; $P = 0.002$) (Table 1). All the patients on CNI therapy remained on CNI-based immunosuppression regimen while patients started on SRL after OLT continued to be on the same immunosuppressive regime in 70.5% at 1 year, 61.6% at 2 years, 66.7% at 3 years, 62.2% at 4 years and 69.3% at 5 years.

Table 1. Demographic and clinical characteristics of the study population.

Variable	Sirolimus group (*n* = 252)	Control group (*n* = 291)	*P* value
Age, years (mean, SD)	49.2 (±10.3)	48.5 (±11.5)	NS
Follow-up period of the cohort, in years (mean, SD)	3.9 (±0.96)	7.1 (±3.8)	*P* = 0.001
Gender			
Males, *n* (%)	167 (66)	170 (59)	NS
Females, *n* (%)	85 (34)	121 (41)	NS
MELD score at the time of transplantation (mean, SD)	15.6 (±4)	15.9 (±11)	NS
Living donor graft recipients, *n* (%)	3 (1.2)	2 (0.6)	NS
Primary indication for transplantation, *n* (%)			
HCV (22 patients had HCC in addition to HCV induced liver failure)	62 (24.6)	61 (20.9)	NS
HCV and ETOH	39 (15.4)	34 (11.6)	NS
ETOH	31 (12.3)	36 (12.3)	NS
PSC	30 (11.9)	37 (12.7)	NS
HCC	29 (11.5)	1 (0.3)	0.003
PBC	22 (8.7)	14 (4.8)	NS
Cryptogenic	12 (4.7)	16 (5.4)	NS
HBV	–	8 (2.7)	NS
Fulminant hepatic failure	–	9 (3)	NS
Autoimmune	–	25 (8.5)	0.002
Other causes	10.7 (27)	50 (17.1)	NS

HCV, hepatitis C Virus; ETOH, alcoholic cirrhosis; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis.

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Primary endpoints of the study

Patient survival rate

No statistical difference was seen in patients' and grafts' survival between the two groups. As represented in Fig. 2, patient survival rates between SRL and CNI group were: 95% vs. 94% at 6 months, 92% vs. 91% at 12 months, 86% vs. 83% at 3 years and 84% vs. 81% at 5 years.

Graft survival rate

Long-term graft survival rate was similar in the two groups as no grafts were lost because of acute or chronic rejection in either cohort. Acute HAT requiring retransplantation was more frequently observed in patients on CNI-based immunosuppression regimen (5.8% vs. 1.2%; $P = 0.004$) and only one patient underwent retransplantation for primary graft nonfunction in each group (P = NS).

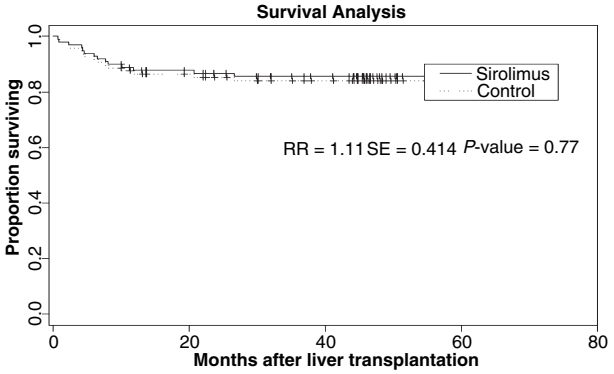

Figure 2 Kaplan–Meier actuarial survival curve of the two cohorts of patients: no statistical difference in the survival probability was observed between the group on SRL-based immunosuppression and the control.

Rejection episodes

Within the first year after OLT, 46.7% of patients on SRL experienced at least one episode of acute cellular rejection in comparison to 56.6% of the control group ($P = 0.003$; Table 2). Although no grafts were lost because of rejection in either group, there was a statistical difference between the two cohorts as the average number of episodes of acute cellular rejection per patient were higher in the CNI group (0.5 vs. 0.7; $P = 0.001$).

Vascular complications

The overall incidence of vascular complications (5.5% vs. 9.2%) was similar in the two groups while HAT was observed more frequently in patients on CNI (5.8% vs. 1.2%; $P = 0.004$) (Table 3). All the subjects who developed HAT underwent re-transplantation and survived. Among all the 252 patients in the SRL group, portal vein thrombosis (PVT) occurred in two recipients (0.8%). One individual was managed conservatively by anticoagulation therapy while the second patient required retransplantation. Among patients in the SRL group, minor pulmonary embolism occurred in four subjects (1.5%) and were treated with systemic anticoagulation, one hepatic artery pseudo-aneurysm required surgical repair (0.4%) and one portal vein stenosis was successfully dilated by percutaneous angioplasty. No statistical difference between the two groups was shown for these adverse vascular events.

Wound complications

There was no difference in the overall incidence of wound complications between the SRL (15%) and CNI group (11.6%) as reported in Table 3. Severe perioperative wound infections requiring debridement in the surgical theater were observed in 14 individuals (5.5%) in the SRL

Table 2. Summary of all episodes of acute cellular rejection occurring during the first year after liver transplantation.

Variable	Sirolimus group (ratio, %)	CNI group (ratio, %)	*P* value
Rejection episodes during the first postoperative year			
Total number of patient alive at 1 year	218/240 (90.8)	95/111 (85.6)	NS
Percentage of patients who developed acute cellular rejection in the first year after OLT	102/218 (46.7)	56/95 (58.9)	0.003
Average number of episodes of acute cellular rejection per patient	131/240 (0.545)	80/111 (0.720)	0.001
Patients with biopsy proven rejection (percentage, number of patients)	78/240 (32.5)	49/111 (44.1)	0.03
Patients with rejection clinically diagnosed (percentage, number of patients)	24/240 (10)	7/111 (6.3)	NS
Patients with only one episode of rejection (percentage, number of patients)	73/240 (30.4)	32/111 (28.8)	NS
Patients with two episodes of rejection (percentage, number of patients)	26/240 (10.8)	18/111 (16.2)	NS
Patients with more than two episodes of rejection (percentage, number of patients)	3/240 (1.25)	4/111 (3.6)	NS
Patients with steroid-resistant rejection (percentage, number of patients)	7/240 (2.9)	5/111 (4.5)	NS
Graft lost because of rejection (percentage, number of patients)	0	0	NS

Data available only from the cohort of patients ($n = 111$) who underwent liver transplantation at the University of Alberta (percentages of all the values were calculated respectively to the number of patients in this inception cohort).

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Table 3. Summary of vascular, bile duct anastomosis, wound complications and infections that occurred perioperatively in the group of patients on SRL-based immunosuppression and in the control group.

Adverse events	Sirolimus group (ratio, %)	CNI group (ratio, %)	*P* value
Vascular adverse events	14/252 (5.5)	27/291 (9.2)*	NS
Hepatic artery thrombosis	3/252 (1.2)	17/291 (5.8)*	0.004
Portal vein thrombosis	2/252 (0.8)	2/111 (1.8)	NS
Pulmonary embolism	4/252 (1.5)	0	NS
Hepatic artery stenosis	4/252 (1.5)	5/111 (4.5)	NS
Hepatic artery pseudoaneurysm	1/252 (0.4)	0	NS
Portal vein anastomotic stenosis	1/252 (0.4)	1/111 (0.9)	NS
Hepatic vein thrombosis	0	1/111 (0.9)	NS
Perioperative wound healing complications	38/252 (15)	34/291 (11.6)*	NS
Wound infection requiring surgical revision in the operating room	14/252 (5.5)	0	0.004
Wound dehiscence requiring emergent surgical repair	2/252 (0.8)	2/111 (1.8)	NS
Abdominal incisional hernia (within the first year after transplantation)	22/252 (8.7)	8/111 (7.2)	NS
Abdominal incisional hernia requiring operative repair within the first 5 years after transplantation	50/252 (19.4)	16/111 (14.1)	NS
Bile duct complications (percentage, number of patients)	49/252 (19.4)	54/291 (18.5)*	NS
Bile duct anastomotic leak	20/252 (7.9)	27/291 (9.2)*	NS
Bile duct clinically significant anastomotic stenosis	29/252 (11.5)	43/111 (14.7)	NS
Opportunistic infections occurring during the first 30 days or during the same hospital stay after liver transplantation	101/252 (40)	41/111 (36.9)	NS
Bacteremia	28/252 (11.1)	13/111 (11.7)	NS
Pneumonia	26/252 (10.3)	11/111 (9.9)	NS
Urinary tract infection	23/252 (9.1)	8/111 (7.2)	NS
Clostridium difficile colitis	12/252 (4.7)	7/111 (6.3)	NS
Candida albicans fungemia	1/252 (0.4)	1/111 (0.9)	NS
Aspergillus pneumonia	0	1/111 (0.9)	NS
Other infections	11/252 (4.3)	0	0.004
Late opportunistic infections (after discharge and within the first 6 months after liver transplantation) (total number of patients on Sirolimus: 226) (total number of patients in the control group: 100)	42/226 (18.5)	13/100 (13)	NS
Bacteremia	10/226 (4.4)	2/100 (2)	NS
Pneumonia	6/226 (2.6)	4/100 (4)	NS
Urinary tract infection	5/226 (2.2)	6/100 (6)	NS
Clostridium difficile colitis	14/226 (6.1)	1/100 (1)	0.001
Other infections	7/226 (3.0)	0	0.02
Cytomegalovirus infection (within the first 6 months after liver transplant) (total number of patients: 226) (total number of patients in the control group: 109)	6/226 (2.6)	3/109 (2.7)	NS
Herpes Virus pneumonia (within the first 6 months after liver transplant)t (total number of patients: 226) (total number of patients in the control group: 109) (percentage, number of patients)	0	0.9 (1/109)	NS
Post-transplant lymphproliferative disease	1/252 (0.4)	2/111 (1.8)	NS

*Data available for the entire cohort of patients in the control group (n = 291) who underwent liver transplantation at the University of Alberta and at the University of Colorado. The remaining values of the control group are reported only for patients operated at the University of Alberta.

group versus none in the control group (P = 0.004). Fascial dehiscences were observed with similar incidence in two recipients (0.8%) receiving SRL immunosuppression and two patients (1.8%) on CNI therapy. Within the first 5 years after OLT, symptomatic incisional hernias that required repair were observed in 50 of the 252 patients (19.4%) on SRL and in 16 of 111 patients (14.1%) transplanted at the UOA and on CNI immunosuppression (P = NS).

Bile duct complications

Bile duct complications (stricture or leak) that required interventions (ERC or PTC) occurred in 19.4% of patients on SRL and in 18.5% of patients on CNI

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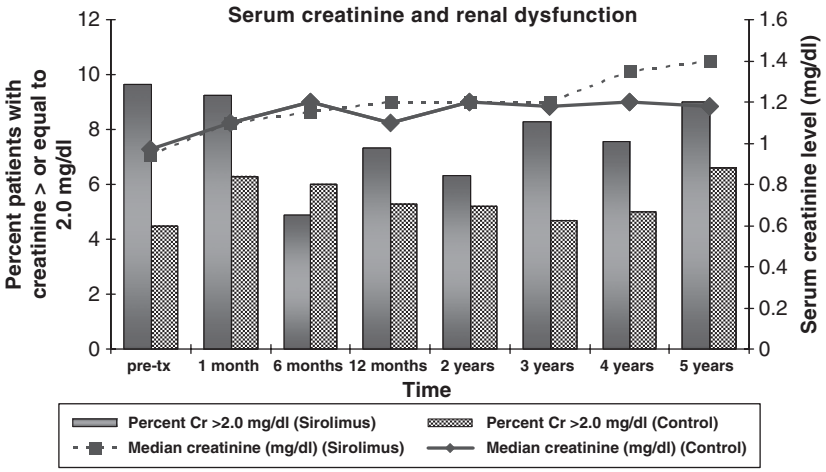

Figure 3 Graphical representation of the interval variations of serum creatinine levels and the percentage of patients with serum creatinine levels equal or above 2 mg/dl in the study population during the time interval of 5-year post liver transplantation. The serum creatinine levels of patients who developed post-transplantation renal failure requiring dialysis were excluded at the time when renal replacement therapy was started.

Table 4. Summary of interval variations of the levels of hematocrit, white blood cells and platelet count, cholesterol and triglycerides of the studied population during the 5-year post liver transplantation. Serum cholesterol levels were similar in the two groups except at the second year postoperatively when patients on sirolimus experienced statistically higher levels of cholesterolemia. Similarly, patients on sirolimus had significant higher levels of serum triglycerides in comparison to the control group during the entire period of follow-up. The gray color of cells represents values that reached statistical significant difference between the two groups ($P < 0.05$).

Parameter	Group	1 month	6 months	12 months	2 years	3 years	4 years	5 years	*P* value
Hct, % (SD)	Sirolimus	31.9 (5.2)	37.8 (6.0)	39.3 (6.4)	40.8 (5.5)	42.1 (5.3)	41.7 (6.2)	39.7 (6.5)	NS
	Control	31.3 (4.6)	36.6 (4.7)	36.5 (5.7)	37.6 (5.1)	38.7 (5.5)	39.1 (4.5)	38.9 (5.8)	
WBC, 10^9/l (SD)	Sirolimus	6.1 (3.6)	4.7 (1.9)	5.1 (4.6)	5.2 (2.0)	5.4 (1.8)	6.6 (2.8)	6.0 (3.2)	NS
	Control	7.3 (3.0)	5.3 (2.1)	5.0 (1.8)	5.0 (1.7)	5.0 (1.5)	5.6 (1.7)	5.6 (2.0)	
Platelets, 10^9/l (SD)	Sirolimus	250.0 (154)	178.9 (87)	186.7 (83)	191.6 (85)	191.8 (78)	198.2 (87)	193.0 (73)	NS
	Control	212.3 (114)	174.0 (82)	174.6 (90)	189.9 (92)	198.8 (80)	213.6 (105)	215.4 (95)	
Cholesterol, mg/dl (SD)	Sirolimus	163.7 (44)	182.2 (55)	178.1 (44)	180.8 (45)	184.5 (50)	190.1 (59)	182.1 (47)	$P < 0.05$
	Control	158.0 (47)	181.2 (47)	170.5 (44)	163.1 (39)	178.8 (43)	177.1 (40)	170.3 (47)	
Triglycerides, mg/dl (SD)	Sirolimus	198.3 (112)	218.0 (182)	193.6 (117)	196.1 (127)	219.1 (144)	242.8 (163)	216.6 (175)	$P < 0.05$
	Control	161.9 (57)	152.6 (94)	145.9 (82)	125.7 (74)	152.3 (79)	152.2 (81)	139.1 (68)	

(Table 3). There was no statistical difference between the two groups. The majority of strictures were treated by endoscopic dilatation and stenting, while leaks were treated by percutaneous drainage and/or reconstruction with hepaticojejunostomy.

Secondary endpoints

Opportunistic infections

The rate of opportunistic infections was similar in the two groups (40% vs. 36.9%, P = NS) (Table 3). Symptomatic cytomegalovirus infection with clinical manifestations was observed in six individuals (2.6%) in the SRL group during the first 6 months period and in only one additional patient after the first year post OLT. Post-transplant Post-transplant lymphoproliferative disease in the SRL group was observed in one patient more than 1 year postsurgery and no patient was diagnosed with SRL-induced pneumonitis. In the control group, post-transplant lymphoproliferative disease (PTLD) was diagnosed in one patient in the first 3 months after OLT and one at 14 months after surgery.

Metabolic outcomes

Renal function

No statistical difference between the two groups was seen during any of the time-intervals analysed (Fig. 3). Among all 252 patients on SRL, three individuals (1.2%) developed acute renal failure prior to OLT and 21 patients (8.8%) required temporary hemodialysis (HD) in the perioperative period. Only five patients (2.2%) still required HD at 6 months. After excluding these patients, the preoperative mean serum creatinine level of patients on SRL was 0.9 mg/dl (SD = 1.21) and 9.6% had serum creatinine above 2.0 mg/dl. The prevalence of renal

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dysfunction (serum creatinine ≥2.0 mg/dl) among the surviving patients declined from 9.8% at 1 month post OLT to 4.8% at 6 months and then remained in the range of 7% (12 months) to 9% (60 months). A similar trend was observed for patients on CNI: three (1%) patients required HD prior to OLT. After excluding these patients, the mean preoperative serum creatinine level was 1.0 (SD = 0.4) and five (1.7%) required temporary HD during the first month after surgery. The prevalence of renal dysfunction (serum creatinine ≥2.0 mg/dl) among patients on CNI declined from 6.2% at 1 month post OLT to 6% at 6 months and then remained in the range of 5.4% (12 months) to 6.9% (60 months) during the follow-up period.

Bone marrow function

Bone marrow function was similar for patients on SRL in comparison to the control group (Table 4). Mean platelet count declined during the first 6 months after OLT but remained stable afterwards without any significant difference between the two groups. The mean white blood count decreased during the first 6 months after OLT and then remained stable in the range of 5–6 $\times$ 10^9 per liter. Similarly, the hematocrit level progressively increased during the first 3 years after OLT and then remained quite constant with a mean value of 35% in both cohorts. None of the patients required recombinant erythropoietin or granulocyte stimulating factor injections beyond 3 months post OLT.

Lipid profile

Significant difference in the triglyceride profile was observed between patients on SRL and the control group. As represented in Table 4, patients on SRL had higher blood levels of triglycerides although both groups were equally exposed to medical therapy (statins and/or fibrates) for hyperlipidemia as soon as the values of serum lipid levels resulted abnormally high as recommended by the American Association of Heart Diseases and the American College of Cardiology [17] (Fig. 4). No statistical difference was noted for serum cholesterol levels between the two cohorts except after 2 years when patients on SRL had significantly higher levels of cholesterol than the control group. With the appropriate introduction of statins and/or fibrates, both groups had similar serum lipid profiles during the 5-year follow-up.

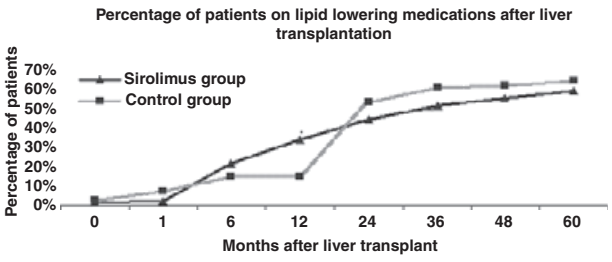

Figure 4 Graphical representation of the percentage of patients on lipid-lowering oral medications after liver transplantation. No statistical difference was observed between patients on SRL-based immunosuppression and the control group except at the 12-month period.

Discussion

The use of SRL in OLT patients has not been approved in the United States because a phase II controlled study has suggested an increased risk of HAT, graft loss and patient death. Nevertheless, up to 15% of OLT recipients in the USA receive SRL within the first year after surgery as it is often used as a CNI-sparing agent, especially in cases of CNI toxicity [27]. Currently, the experience of using SRL in OLT recipients early after surgery seems limited to a few transplant centers and there is only a modest body of literature on its effectiveness and safety as a de novo immunosuppression medication in OLT recipients.

The primary aim of this study was to assess the outcomes of a large cohort of recipients who received SRL in the first 24 h after OLT and continued for at least 6 months afterwards. The comparison of the results was made with a control group of patients treated with standard CNI therapy at the same centers and during the same period. To our knowledge, this is the largest observational study assessing the outcomes of de novo SRL immunosuppression after OLT and most likely it would not be repeated after the FDA issued the black box label [7,13,14,28,29]. Watson [10] *et al.* were the first to describe the use of SRL as primary immunosuppression in OLT. Chang *et al.*subsequently explored the feasibility of converting stable OLT recipients affected by CNI toxicity from CyA or TAC to SRL and reported no significant adverse events in the process [9]. Other authors described similar experiences and observed benefits of using SRL such as prevention of renal dysfunction, reduced neurotoxicity and reduced steroid-resistant allograft rejections [30–33]. Nevertheless, the experience with SRL in OLT recipients in many transplant centers has been modest [34,35] with the majority of programs in the United States, Europe and other countries viewing the use of CNIs as essential for the success of OLT [36,37]. TAC, and less so CyA, are the most commonly used CNI immunosuppressant drugs for solid organ transplant recipients and provide excellent graft- and patient survival rates [38]. However, renal dysfunction or failure [39], hypertension, diabetes, hyperlipidemia and osteoporosis are major side-effects directly related to

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the time of exposure and blood concentration of these drugs [39–41].

At the University of Alberta and the University of Colorado, the use of SRL was implemented in a substantial number of patients undergoing OLT prior to the FDA black box label and in selected patients thereafter to reduce side-effects related to CNI therapy, or as an antitumor agent in those with hepatocellular carcinoma [28,42,43]. As SRL has a different safety profile than CNI, the possible advantages of its use must be weighed against a range of other side-effects such as peripheral swelling, joint pain, wound infections, hyperlipidemia, oral and gastrointestinal ulcerations [44], dermatitis, interstitial pneumonitis [45–47] and possibly vascular thrombosis.

In our experience, patients on SRL had survival and rejection rates, graft functions and infection rates comparable to the control group and confirmed the results of other previous smaller observational studies [14,34,48,49]. One of the most controversial aspects of using SRL in OLT recipients is the potential higher risk of graft loss because of HAT [14]. Our findings did not confirm an increased thrombotic risk for these patients and supported the results reported by other investigators who used SRL after OLT [14,28,50]. It is known that HAT complicates 4–15% of OLT and occurs more frequently after pediatric OLT [51,52]. Several technical and congenital conditions have been found to increase the risks of HAT: dissection of the hepatic arterial wall, technical imperfections with the anastomosis, celiac artery stenosis, hypercoagulable state, transplantation for primary sclerosing cholangitis, aberrant arterial anatomy, back table arterial reconstruction of the allograft, and high-resistance microvascular arterial outflow caused by rejection or severe ischemia-reperfusion injury (IRI) [53–56]. In our experience, thrombotic vascular complications occurred in 5.5% of patients on SRL and in 9.2% of patients on CNI (P = NS). Contrary to our expectations, HAT occurred in 1.2% of patients on SRL and in 5.8% of patients on CNI ($P = 0.004$). One of the possible explanations of these findings is that the group treated with CNI had a significantly higher percentage of patients with autoimmune diseases that are well known to be associated with hypercoagulability and risk of thrombosis. Unfortunately, the small number of patients who developed this complication in each group did not allow us to perform any further statistical analysis to explore this hypothesis further [56].

Over the last two decades, patient- and graft survival rates have improved significantly. Recent literature reports that 75–85% of all individuals undergoing OLT are alive at 5 years independent of the immunosuppression regimen used [57–59]. The longer life-expectancy of these patients demand a careful evaluation of the spectrum of all the long-term side-effects of immunosuppression as they have become the main cause of death with functioning grafts. Epidemiological studies have shown that chronic kidney disease is a predisposing factor for higher morbidity and mortality in transplant recipients [60,61] as well as in the general population [62]. Therefore, immunosuppressive medications without renal toxicity are very attractive. Published clinical studies indicate improved renal function after conversion from CNI to SRL in the first 1–6 months after renal or OLT [33,63,64]. In our study, both groups experienced improved renal function during the first year after transplant and relative stability during the following years possibly because of the resolution of preoperative hepatorenal syndrome. It is important to note that these outcomes were seen in patients transplanted in the pre-MELD era, a time when renal dysfunction was much less common in OLT recipients, and perhaps partially explaining the excellent long-term renal function observed in both groups of patients.

Sirolimus (SRL) has been associated with a negative effect on wound healing [65–68] because of its antifibrotic effects [69]. As in previous reports [70,71], we observed a rather high incidence of incisional hernias requiring surgical revision associated with the use of SRL. Severe perioperative wound infections occurred in 5.8% of patients and wound dehiscences in 0.8%. Symptomatic incisional hernias requiring surgical repair occurred in 50 of 252 patients (19%) during the first 5-year post OLT vs. 14% in the control group. At both transplant centers, it was felt that there was no need to discontinue SRL therapy in the pre- and postoperative period for incisional hernia repairs. Although this difference did not reach statistical power, the results of this study support the finding of other authors who have reported up to 15–17% of patients with incisional hernias requiring surgical therapy after OLT [34,51,52] and some delayed wound healing with SRL [65].

There is limited knowledge regarding the incidence of bile duct complications because of poor healing in patients undergoing OLT and treated with SRL. In this study, the rate of bile duct anastomotic complications was 19.4%, which is comparable to patients treated with CNI [34]. More importantly, few of the adverse events involving the bile duct anastomosis had long-term effects that contributed to graft failure.

In contrast to the previous clinical trials of OLT recipients treated with SRL-based immunosuppression, we saw no increased risk of infection in this group. Overall, the rate of infection in patients on SRL was quite low: bacteremia 11%, pneumonia 10.3%, fungal infection 0.4%, CMV 2.6%, and PTLD 0.4%. None was higher than the

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CNI-treated control group, nor do the infections rates appear higher in comparison to published experience [70]. These findings might be attributable to the lower level of SRL used at our transplant centers, or the combination of lower SRL blood levels and the fact that other immunosuppressive medications such as steroids were tapered relatively quickly. Contrary to other observational studies of solid organ transplant recipients where the incidence of SRL-induced pneumonitis ranged from 2% [72] to 11% [73,74], we did not observe any case. We suspect that this might be as a result of several possibilities. One possible explanation is attributable to the difficulty of diagnosing interstitial pneumonitis resulting from SRL in the early postoperative period as the clinical and radiological presentations are similar to infective pneumonia that occurs much more frequently [75]. Another explanation might be that during the period of this study, the association between exposure to SRL and interstitial pneumonitis had not yet become established and therefore the clinical diagnosis of this condition might have been misinterpreted as infective pneumonia.

Dyslipidemia is perhaps the most common metabolic side-effect of SRL and it may not be dose-dependent [76]. Clinical studies in renal transplantation have demonstrated that up to 80% of patients on SRL have hypercholesterolemia [77,78]. Similar to the renal transplant literature, in OLT recipients treated with SRL, the incidence of hypercholesterolemia has been reported to be as high as 44% [29]. In comparison to previous studies, the lipid profile of our patients showed modest levels of hyperlipidemia that might have been attributable to the combination of maintaining lower blood levels of SRL, and/or the reduced use of steroids in addition to the introduction of statins and/or fibrate medications for all patients with elevated serum lipid profile.

Sirolimus (SRL)-associated bone marrow suppression is attributable to inhibition of specific cytokines and vascular growth factors [68,79,80]. In our study, SRL did not significantly affect platelet or white blood counts; they remained in the range of 160–190 000/mm^3 and 4000–6000/mm^3 respectively over time without frequent need for granulocyte stimulating growth factor injection. Similarly, the level of hematocrit remained quite stable (31–40%) although oral iron supplementation was prescribed for all patients who had shown evidence of suppressed erythropoietic function. Anemia was less commonly a problem in Denver, likely a result of the altitude-induced higher baseline level of hematocrit.

As in many retrospective observational studies, there are several inherent weaknesses of this study mostly attributable to the lack of randomization of patients. Although the immunosuppression protocols used for subjects treated with SRL were similar at both medical centers, selection and treatment bias could not be avoided without random allocation. As in every retrospective analysis there is the risk of introducing sampling, selection and other bias as the groups of patients assembled for the study differ in ways other than the factors under investigation. Although, no significant baseline differences were seen between recipients on CNI-based immunosuppression or SRL-based one, there was heterogeneity in etiology of cirrhosis, pre- and postoperative care provided at the two participating medical centers. For example, in both centers, the protocols employed for the use of SRL were started early after OLT and continued for at least six consecutive months. On the other hand, the use of steroids and the blood level of immunosuppressive medications were not uniform. Another important limitation of this study is that the majority of patients were enrolled prior to the introduction of the MELD scoring system for the allocation of cadaveric grafts. The mean MELD score at the time of transplantation for patients enrolled in this study was only 15, this being the average MELD score for patients transplanted in the pre-MELD era and this value is significantly lower than the average score of patients undergoing OLT in recent years [81,82]. Higher MELD scores are associated with more advanced liver and other organs dysfunction. Therefore, the incidence of pre- and post-transplant renal insufficiency, infections, and wound healing problems observed in these two cohorts may apply only to patients with relatively preserved hepatic and renal function.

Despite these limitations, our study has the strength of being one of the largest observational studies on the use of SRL in OLT recipients treated in North America. After the black box label, the experience of SRL in OLT has been quite modest and most likely a similar study will not be feasible any time in the near future. Our findings can not overturn the results of randomized trials, but they suggest that for patients with moderate MELD scores at the time of OLT, the use of SRL alone or in conjunction with low-dose CNI may be safe and effective as rejection rates, graft losses and patient survivals were similar in both the groups. Nevertheless, we recognize the limitations of our study, and wish that new randomized controlled trials are performed to test the effectiveness and safety of promising new m-TOR inhibitors for OLT recipients.

Authorship

NK, JFT, KB and MM: participated in research design. MM, NK and JFT: participated in the writing of the paper. NK, KB, GM, JAS, DB, JFT and MM: participated in the performance of the research. MM, NK and JFT: participated in data analysis.

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References

1. Starzl TE, Klintmalm GB, Porter KA, Iwatsuki S, Schroter GP. Liver transplantation with the use of cyclosporin A and prednisone. *N Engl J Med* 1981; **305**: 266.
2. Yi NJ, Suh KS, Lee HW, *et al.* Improved outcome of adult recipients with a high model for end-stage liver disease score and a small-for-size graft. *Liver Transpl* 2009; **15**: 496.
3. Gerhardt T, Terjung B, Knipper P, *et al.* Renal impairment after liver transplantation - a pilot trial of calcineurin inhibitor-free vs. calcineurin inhibitor sparing immunosuppression in patients with mildly impaired renal function after liver transplantation. *Eur J Med Res* 2009; **14**: 210.
4. Rifai K, Kirchner GI, Bahr MJ, *et al.* A new side effect of immunosuppression: high incidence of hearing impairment after liver transplantation. *Liver Transpl* 2006; **12**: 411.
5. Dumortier J, Bernard S, Bouffard Y, Boillot O. Conversion from tacrolimus to cyclosporine in liver transplanted patients with diabetes mellitus. *Liver Transpl* 2006; **2**: 659.
6. Anonymous. The U.S. Multicenter FK 506 Liver Study Group. Comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med* 1994; **323**: 1110.
7. Lo A, Egidi MF, Gaber LW, *et al.* Comparison of sirolimus-based calcineurin inhibitor-sparing and calcineurin inhibitor-free regimens in cadaveric renal transplantation. *Transplanatation* 2004; **77**: 1228.
8. Groth CG, Backman L, Morales JM, *et al.* Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. *Transplantation* 1999; **67**: 1035.
9. Chang GJ, Mahanty HD, Quan D, *et al.* Experience with the use of sirolimus in liver transplantation–use in patients for whom calcineurin inhibitors are contraindicated. *Liver Transpl* 2000; **6**: 734.
10. Watson CJ, Friend PJ, Jamieson NV, *et al.* Sirolimus: a potent new immunosuppressant for liver transplantation. *Transplantation* 1999; **67**: 505.
11. Neff GW, Montalbano M, Tzakis AG. Ten years of sirolimus therapy in orthotopic liver transplant recipients. *Transplant Proc* 2003; **35**(Suppl. 3A): 209S.
12. Trotter JF, Wachs M, Bak T, *et al.* Liver transplantation using sirolimus and minimal corticosteroids (3-day taper). *Liver Transpl* 2001; **7**: 343.
13. Kneteman NM, Oberholzer J, Al Saghier M, *et al.* Sirolimus-based immunosuppression for liver transplantation in the presence of extended criteria for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 1301.
14. Dunkelberg JC, Trotter JF, Wachs M, *et al.* Sirolimus as primary immunosuppression is not associated with hepatic artery thrombosis or wound complications. *Liver Transpl* 2003; **9**: 463.
15. Trotter JF, Levi M, Steinberg T, Lancaster J. Absence of Pneumocystis jiroveci pneumonia in liver transplantation recipients receiving short-term (3-month) prophylaxis. *Transpl Infect Dis* 2008; **10**: 369.
16. Trotter JF, Wallack A, Steinberg T. Low incidence of cytomegalovirus disease in liver transplant recipients receiving sirolimus primary immunosuppression with 3-day corticosteroid taper. *Transpl Infect Dis* 2003; **5**: 174.
17. http://www.americanheart.org (last accessed on 07/10/2009)
18. Freeman RB Jr, Wiesner RH, Harper A, *et al.* UNOS/OPTN Liver Disease Severity Score, UNOS/OPTN Liver and Intestine, and UNOS/OPTN Pediatric Transplantation Committees. The new liver allocation system: moving toward evidence-based transplantation policy. *Liver Transpl* 2002; **8**: 851.
19. Colantonio DA, Borden KK, Clarke W. Comparison of the CEDIA and MEIA assays for the measurement of sirolimus in organ transplant recipients. *Clin Biochem* 2007; **40**: 680.
20. Luna CM, Videla A, Mattera J, *et al.* Blood cultures have limited value in predicting severity of illness and as a diagnostic tool in ventilator-associated pneumonia. *Chest* 1999; **116**: 1075.
21. Guay DR. Contemporary management of uncomplicated urinary tract infections. *Drugs* 2008; **68**: 1169.
22. Swoboda SM, Dixon T, Lipsett PA. Can the clinical pulmonary infection score impact ICU antibiotic days? *Surg Infect* 2006; **7**: 331.
23. Stratta RJ, Shaeffer MS, Markin RS, *et al.* Cytomegalovirus infection and disease after liver transplantation. An overview. *Dig Dis Sci* 1992; **37**: 673.
24. Langnas AN, Marujo W, Stratta RJ, Wood RP, Shaw BW Jr. Vascular complications after orthotopic liver transplantation. *Am J Surg* 1991; **161**: 76.
25. Wojcicki M, Milkiewicz P, Silva M. Biliary tract complications after liver transplantation: a review. *Dig Surg* 2008; **25**: 245.
26. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for Prevention of Surgical Site Infection, 1999. Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. *Am J Infect Control* 1999; **27**: 97.
27. Zaghla H, Selby RR, Chan LS, *et al.* A comparison of sirolimus vs. calcineurin inhibitor-based immunosuppressive therapies in liver transplantation. *Aliment Pharmacol Ther* 2006; **23**: 513.
28. Toso C, Meeberg GA, Bigam DL, *et al.* De novo sirolimus-based immunosuppression after liver transplantation for hepatocellular carcinoma: long-term outocomes and side effects. *Transplantation* 2007; **83**: 1162.
29. Trotter JF, Wachs ME, Trouillot TE, *et al.* Dyslipidemia during sirolimus therapy in liver transplant recipients occurs with concomitant cyclosporine but not tacrolimus. *Liver Transpl* 2001; **7**: 401.

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30. Perdigoto R, Sequeira C, Tome L, *et al.* Sirolimus: efficacy in liver transplant patients with nephrotoxicity and renal insufficiency. *Transplant Proc* 2007; **39**: 2519.
31. Watson CJ, Gimson AE, Alexander GJ, *et al.* A randomized controlled trial of late conversion from calcineurin inhibitor (CNI)-based to sirolimus-based immunosuppression in liver transplant recipients with impaired renal function. *Liver Transpl* 2007; **13**: 1694.
32. Neff GW, Montalbano M, Slapak-Green G, *et al.* A retrospective review of sirolimus (Rapamune) therapy in orthotopic liver transplant recipients diagnosed with chronic rejection. *Liver Transpl* 2003; **9**: 477.
33. Dominguez J, Mahalati K, Kiberd B, McAlister VC, MacDonald AS. Conversion to rapamycin immunosuppression in renal transplant recipients: report of an initial experience. *Transplantation* 2000; **70**: 1244.
34. 2003 Organ Procurement and Transplantation Network Scientific Registry of Transplant Recipients Annual Report: Transplant Data 1993–2002. US Department of Health and Human Services, Health Resources and Services Administration, Special Program Bureau, Division of Transplantation; United Network of Organ Sharing; University Renal Research and Education Association (Table 9.6). Available at: http://www.ustransplant.org/cgi-bin/ar?p=data_tables_10.htm&y=2–3.
35. Kaufman D, Shapiro R, Lucey MR, Cherikh WS, Bustami RT, Dyke DB. Immunosuppression: practice and trends. *Am J Transplant* 2004; **4**(Suppl. 9): 38.
36. Chan KL, Saing H, Fan ST, Wei WI, Ng IO, Tsoi NS. Cyclosporine-based immunotherapy for pediatric liver transplantation in Hong Kong. *Transplant Proc* 1996; **28**: 1302.
37. Barcena R, Lopez-San Roman A, Garcia M, Ruiz Del Arbol L, De Vicente E, Candela A. Immunosuppression in liver transplantation: a European survey. *Transplant Proc* 1999; **31**: 2427.
38. Fung J, Kelly D, Kadry Z, Patel-Tom K, Eghtesad B. Immunosuppression in liver transplantation. Beyond calcimeurin inhibitors. *Liver Transpl* 2005; **1**: 267.
39. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med* 2003; **349**: 931.
40. McCauley J, Fung JJ, Brown TH, *et al.* Renal function after conversion from CsA to FK506 in liver transplant patients. *Transplant Proc* 1991; **23**: 3148.
41. Faenza S, Santoro A, Mancini E, *et al.* Acute renal failure requiring renal replacement after orthotopic liver transplantation. *Transplant Proc* 2006; **38**: 1141.
42. Yang YJ, Li LX, He Q, *et al.* Sirolimus as primary immunosuppressant for calcineurin inhibitor-related renal insufficiency after liver transplantation. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 376.
43. McAlister VC, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS. Sirolimus-tacrolimus combination immunosuppression (letter). *Lancet* 2000; **355**: 376.
44. Molinari M, Al-Saif F, Ryan AE, *et al.* Sirolimus-induced ulceration of the small bowel in islet transplant recipients – Reports of two cases. *Am J Transplant* 2005; **5**: 2799.
45. Whiting PH, Adam BJ, Woo J, Hasan NU, Thomson AW. The effect of rapamycin on renal function in the rat: a comparative study with cyclosporine. *Toxicol Lett* 1991; **58**: 169.
46. Guilbeau JM. Delayed wound healing with sirolimus after liver transplant. *Ann Pharmacother* 2002; **36**: 1391.
47. Kaplan MJ, Ellis CN, Bata-Csorgo Z, Kaplan RS, Endres JL, Fox DA. Systemic toxicity following administration of sirolimus (formerly rapamycin) for psoriasis: association of capillary leak syndrome with apoptosis of lesional lymphocytes. *Arch Dermatol* 1999; **135**: 553.
48. Silva MA, Jambulingam PS, Gunson BK, *et al.* Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl* 2005; **12**: 146.
49. Zaghla H, Selby RR, Chan LS, *et al.* A comparison of sirolimus vs. calcineurin inhibitor-based immunosuppressive therapies in liver transplantation. *Aliment Pharmacol Ther* 2006; **23**: 513.
50. McAlister VC, Peltekian KM, Malatjalian DA, *et al.* Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. *Liver Transpl* 2001; **7**: 701.
51. Wozney P, Zajko AB, Bron KM, *et al.* Vascular complications after liver transplantation: a 5-year experience. *AJR Am J Roentgenol* 1986; **147**: 657.
52. Segel MC, Zajko AB, Bowen A, *et al.* Hepatic artery thrombosis after liver transplantation: radiologic evaluation. *AJR Am J Roentgenol* 1986; **146**: 137.
53. Stange B, Glanemann M, Nuessler N, Settmacher U, Steinmueller T, Neuhaus P. Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl* 2003; **9**: 612.
54. Oh C, Pelletier S, Sawyer R, *et al.* Uni- and multi-variate analysis of risk factors for early and late hepatic artery thrombosis after liver transplantation. *Transplantation* 2001; **71**: 767.
55. Pastacaldi S, Teixeira R, Montalto P, Rolles K, Burroughs A. Hepatic artery thrombosis after orthotopic liver transplantation: a review of nonsurgical causes. *Liver Transpl* 2001; **7**: 75.
56. Norman D, Turka L, Young TE. Diagnosis and treatment of hepatic allograft dysfunction. In: *Primer on Transplantation*., 2nd edn. New Jersey: Wiley-Blackwell, 2001: 565.
57. Cross TJS, Antoniades CG, Muiesan P, *et al.* Liver transplantation in patients over 60 and 65 years: an evaluation of long-term outcomes and survival. *Liver Transpl* 2007; **13**: 1382.
58. Dawwas MF, Gimson AE, Lewsey JD, Copley LP, van der Meulen JH. Survival after liver transplantation in the United Kingdom and Ireland compared with the United States. *Gut* 2007; **56**: 1606.
59. Kniepeiss D, Iberer F, Grasser B, Schaffellner S, Tscheliessnigg KH. Sirolimus and mycophenolate mofetil after liver transplantation. *Transpl Int* 2003; **16**: 504.

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60. Stratta P, Canavese C, Quaglia M, *et al.* Postransplantation chronic renal damage in nonrenal transplant recipients. *Kidney Int* 2005; **68**: 1453.
61. Meier-Kriesche HU, Steffen BJ, Hochberg AM, *et al.* Long-term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. *Am J Transplant* 2003; **3**: 68.
62. Jayasena SD, Riaz A, Lewis CM, Neild GH, Thompson FD, Woolfson RG. Outcome in patients with end-stage renal disease following heart or heart-lung transplantation receiving peritoneal diálisis. *Nephrol Dial Transplant* 2001; **16**: 1681.
63. Diekmann F, Waiser J, Fritsche L, Dragun D, Neumayer HH, Budde K. Conversion to rapamycin in renal allograft recipients with biopsy-proven calcineurin inhibitor-induced nephrotoxicity. *Transplant Proc* 2001; **33**: 3234.
64. Fairbanks KD, Eustace JA, Fine D, Thuluvath PJ. Renal function improves in liver transplant recipients when switched from a calcineurin inhibitor to sirolimus. *Liver Transpl* 2003; **9**: 1079.
65. Guilbeau JM. Delayed wound healing with sirolimus after liver transplant. *Ann Pharmacother* 2002; **36**: 1391.
66. Mathis AS. Comment: delayed wound healing with sirolimus after liver transplant. *Ann Pharmacother* 2003; **37**: 453.
67. Troppmann C, Pierce JL, Gandhi MM, Gallay BJ, McVicar JP, Perez RV. Higher surgical wound complication rates with sirolimus immunosuppression after kidney transplantation: a matched-pair pilot study. *Transplantation* 2003; **76**: 426.
68. Montalbano M, Neff GW, Yamashiki N, *et al.* A retrospective review of liver transplant patients treated with sirolimus from a single center: an analysis of sirolimus-related complications. *Transplantation* 2004; **78**: 264.
69. Sousa JE, Costa MA, Abizaid A, *et al.* Lack of neointimal proliferation after implantation of sirolimus-coated stents in human coronary arteries: a quantitative coronary angiography and three-dimensional intravascular ultrasound study. *Circulation* 2001; **103**: 192.
70. Fisher A, Seguel JM, de la Torre AN, *et al.* Effect of sirolimus on infection incidence in liver transplant recipients. *Liver Transpl* 2004; **10**: 193.
71. Wiesner R, for the Rapamune Liver Transplant Study Group. The safety and efficacy of sirolimus and low-dose tacrolimus vs. tacrolimus in de novo orthotopic liver transplant recipients: results from a pilot study [Abstract]. *Hepatology* 2002; **36**: 208A.
72. Roberts RJ, Wells AC, Unitt E, *et al.* Sirolimus-induced pneumonitis following liver transplantation. *Liver Transpl* 2007; **13**: 853.
73. Champion L, Stern M, Israel-Biet D, *et al.* Brief communication: sirolimus-associated pneumonitis: 24 cases in renal transplant recipients. *Ann Intern Med* 2006; **144**: 505.
74. Shaffer D, Langone A, Nylander WA, Goral S, Kizilisik AT, Helderman JHA. Pilot protocol of a calcineurin-inhibitor free regimen for kidney transplant recipients of marginal donor kidneys or with delayed graft function. *Clin Transplant* 2003; **17**(Suppl. 9): 31.
75. Morelon E, Stern M, Israel-Biet D, *et al.* Characteristics of sirolimus-associated interstitial pneumonitis in renal transplant patients. *Transplantation* 2001; **72**: 787.
76. Firpi RJ, Tran TT, Flores P, *et al.* Sirolimus-induced hyperlipidaemia in liver transplant recipients is not dose-dependent. *Aliment Pharmacol Ther* 2004; **19**: 1033.
77. Groth Groth CG, Backman L, Morales JM, *et al.* Sirolimus (rapamycin)-based therapy in human renal transplantation. *Transplantation* 1999; **67**: 1036.
78. Kahan BD, for Rapamune® US Study Group. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection. *Lancet* 2000; **356**: 194.
79. Hong JC, Kahan BD. Sirolimus induced thrombocytopenia and leucopenia in renal transplant recipients: risk factors, incidence, progression, and management. *Transplantation* 2000; **69**: 2085.
80. Nair S, Eason J, Loss G. Sirolimus monotherapy in nephrotoxicity due to calcineurin inhibitors in liver transplant recipients. *Liver Transpl* 2003; **9**: 126.
81. Massicotte L, Beaulieu D, Roy JD, *et al.* MELD score and blood product requirements during liver transplantation: no link. *Transplantation* 2009; **87**: 1689.
82. Medici V, Rossaro L, Wegelin JA, *et al.* The utility of the model for end-stage liver disease score: a reliable guide for liver transplant candidacy and, for select patients, simultaneous hospice referral. *Liver Transpl* 2008; **14**: 1100.

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