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# *Handling sirolimus in clinical practice*

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## HYPERLIPIDEMIA AND CARDIOVASCULAR DISEASE FACTORS

Since the first studies of maintained cyclosporine plus steroid combination, it was found that patients who also received the arm with the highest sirolimus (SRL) doses showed higher cholesterol and triglyceride numbers than patients who received sirolimus at lower doses or azathioprine[1,2] or placebo[2,3]. Attempts have been made to analyze the contribution of this hyperlipidemia to a possible greater post-transplant cardiovascular risk by means of the Framingham index[4], although it can be highlighted that this Framingham index is not a good predictor for the renal transplant recipient population[5], and that it does not consider renal function status[6-8]. Kahan's group retrospectively reviewed their experience with the combination of sirolimus, cyclosporine and steroids[9]. In multivariate analysis the factors associated to the occurrence of hypercholesterolemia were, from the most significant to the least significant: CsA C2 levels, prior pre-transplant cholesterol levels, sirolimus trough levels and the accumulated steroid dose. The factors associated to the occurrence of hypertriglyceridemia were, from the most significant to the least significant: prior pre-transplant triglyceride levels and sirolimus trough levels.

In terms of whether sirolimus is more hyperlipidemic than cyclosporine, studies 207 and 210 comparing cyclosporine to sirolimus at levels that seem extremely high today (20-30 ng/mL), showed that the cholesterol figures were greater in the latter of the two. It is also suggested that hyperlipidemia is level-dependent[10], although these differences were not significant beyond 12 months after the transplant when initial sirolimus levels were attenuated. Similarly, a Cleveland group trial that used basiliximab, mycophenolate and steroids and randomized the patients to receive sirolimus or cyclosporine, showed no differences in terms of cholesterol between both arms[11], although a higher number of patients with sirolimus received hypolipidemic drugs. The type of hyperlipidemia associated to the sirolimus-cyclosporine combination seems to consist of an increase in total cholesterol, LDL cholesterol, apo-B100, apoC-III, the free fatty acid pool and triglycerides, but seemingly not affecting apo A-1 levels. Lipase lipoprotein activity is reduced, although in a manner similar to the cyclosporine with mycophenolate scheme[12-14]. In order to complete the symptoms of lipoprotein alterations, some studies in animal experiments[15,16]and in clinical experience[17] suggest that sirolimus also increases the HDL fraction, which according to Kasiske et al., is the most important lipoprotein fraction alteration in cardiovascular events[18]. Cardiovascular risk analysis of clinical trial 310, in which the combination of cyclosporine with sirolimus was compared to sirolimus at higher levels showed that the tendency for higher hypercholesterolemia in the SRL group was at the expense of both HDL and LDL[19].

Lipoprotein profile of the combination of cyclosporine with sirolimus were suggested to be similar to that observed in insulin resistance situations. There is currently contradictory data concerning the role of sirolimus in glucose metabolism. Araki et al. have studied in a retrospective way the development of postransplant diabetes in their cohorts of patients maintained with cyclosporine, tacrolimus or sirolimus, all plus mycophenolate and steroids. In the multivariate analysis, the treatment with tacrolimus, the weight, the age and the anti-rejection treatment were associated with the new onset development of diabetes[20]. The Bari group has found that when the calcineurin inhibitor is withdrawn and sirolimus begun, insulin resistance increases[21], the latter being correlated with triglyceride increases[21]. However, que Havrdova et al. have found better insulin responses rates with sirolimus than with mofetil mycophenolate[22]. It has recently been shown that S6 kinase, an mTOR effector, would mediate insulin signaling inhibition, such that mice with selective S6K1 deletion would show diet-induced resistance to obesity and a better hypoglycemic response to insulin[23]. As a result it has been suggested that sirolimus (or

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other direct S6K1 inhibitors) could be beneficial in metabolic disorders such as type II diabetes characterized by insulin resistance[24,25]. The relevance of mTOR in obesity control could explain former observations in adult patients with cyclosporine and steroids who showed smaller significant weight increases when they were also treated with sirolimus rather than the placebo. Similarly, the Texas group found that in pediatric patients treated with sirolimus, the BMI seemed to be lower than in those treated with cyclosporine[26]. Clinical experience with sirolimus does not suggest a relevant hyperglycemic effect of sirolimus, and it has even been successfully used in post-transplant glucose intolerances[27]. Unfortunately the prevalence of metabolic syndrome criteria has not been included in clinical studies with sirolimus.

Despite a proven hyperlipidemic effect of sirolimus several characteristics of the drug may make the final cardiovascular profile of sirolimus favorable. Some of this proof is preclinical, such as:

- In APO-E deficient hyperlipidemic mice, it is shown that the administration of sirolimus decreases aortic atheromatous plaque[15,28].
- Sirolimus inhibits in vivo intimal proliferation produced by mechanical damage[29] or immunological damage[30].
- Sirolimus prevents[31] and controls[32] the development of ventricular hypertrophy due to overload in murine models.
- Sirolimus decreases the intracellular accumulation in mesangial cells by increasing expulsion from the cell by means of overexpressing transport proteins, such as ABC A1, and by decreasing entry by means of decreasing LDL and VLDL receptors[33].

Several clinical experiments also suggest a favorable cardiovascular profile:

- In the sirolimus plus cyclosporine combination trial with later withdrawal of cyclosporine, there were no differences in terms of cardiovascular events between both arms after two years of follow-up[34]. Kahan's group did not observe cardiovascular events after 3 years in the group treated with sirolimus compared to the group not treated, despite proof of the hyperlipidemic effect of sirolimus[9].
- The withdrawal of cyclosporine also obtained lower systolic, diastolic and average blood pressure figures from the beginning of the withdrawal of the calcineurin inhibitor[19,35-37]. The withdrawal of tacrolimus from a regimen with sirolimus also tends to improve diastolic blood pressure[38]. A recent meta-analysis of calcineurin inhibitors withdrawal trials in patients maintained on sirolimus confirms these findings[39].
- In heart transplants, the randomized trial that compared sirolimus to azathioprine, both with cyclosporine and steroids, showed better vascular disease rates for the graft measured by intravascular ultrasound in the sirolimus group[40]. It is also able to control vascular disease for the graft once the latter has been established[41].
- Two studies have tested the efficacy of a short oral administration of sirolimus to treat different forms of coronary stenoses[42-44], although others have not found an acceptable risk/benefit ratio[45].

**Recommendations**

Until the role of sirolimus on lipid metabolism and arteriosclerosis pathogenesis has not been firmly established, hyperlipidemia associated to sirolimus must be intensely treated following the recommendations of the III Panel of the National Cholesterol Evaluation Program which aim to reach an LDL figure of less than 130 mg/dl[46] or even those aimed towards the kidney patient population which champion for LDL figures less than 100 mg/dl[47]. No clinically significant interactions between sirolimus and atorvastatin have been observed[48,49]. No episodes of rhabdomyolysis have been reported in the combined use of statins and sirolimus[10], but it is necessary to monitor for the possible occurrence of muscle symptoms[50].

Long-term follow-up for patients taking sirolimus is necessary in order to observe long-term cardiovascular mortality and morbidity. Future de novo and conversion trials with sirolimus must also include the determination of other cardiovascular risk factors, such as C-reactive protein, homocysteine, metabolic syndrome incidence, endothelial dysfunction parameters, insulin resistance indexes, etc.

Given that the response of hyperlipidemia associated to sirolimus is fairly satisfactory, sirolimus cannot be contraindicated in those patients with pretransplant significant hyperlipidemia. On the other hand, although the incidence of hyperlipidemia associated with sirolimus is rather frequent, it does not seem necessary to begin preventive treatment with statins when sirolimus begins.

Preliminary data suggests that SRL with tacrolimus[51] or with mycophenolate mofetil[11] would be less hyperlipidemic than the combination with CsA[17].

## HAEMATOLOGICAL CYTOPENIAS

### Anemia

In sirolimus development trials there seemed to be lower hemoglobin figures in the groups that were most exposed to sirolimus and in the combinations of the latter with an antimetabolite (see table 1).

Study 310 evaluated the elimination of cyclosporine 3 months after the transplant with a triple sirolimus, cyclosporine and steroid regimen[35-37]. During the entire follow-up period up until five years, mean hemoglobin levels were significantly higher in the cyclosporine elimination group compared to the maintenance group with the three drugs despite greater exposure to sirolimus in the first group[52]. This finding could be due to the synergistic effect of the combination of cyclosporine with sirolimus, decreasing cellularity of the bone marrow as has been shown in murine models[16].

With regard to whether the anemia-inducing effect of sirolimus is greater or lesser than that of mycophenolate, a retrospective analysis at Cleveland University compared two transplant cohorts, anemia being more prevalent in the sirolimus group[53]. However, a similar analysis at the Philadelphia University found the opposite[54]. On the other hand, the combination of two drugs with an anemia-inducing effect could enhance their effects. Some studies compared the evolution of the hemoglobin figure in the sirolimus with mycophenolate combination with the cyclosporine with mycophenolate combination, finding a greater tendency for anemia with the first regimen at the beginning of treatment and tending to be identical to one another at the end of the first year[11,55-57].

Anemia associated with sirolimus has been described as aregenerative with high ferritin[58]. Some isolated data suggests that an almost universal decrease in the mean corpuscular volume is observed[55,58,59], as well as the mean corpuscular hemoglobin concentration[60]. Response to treatment with iron does not seem to be effective[53,60]. Decreased hemoglobin figures that required temporary treatment with erythropoietin have frequently been reported in conversions to sirolimus[61], although as well in other postransplant anemia setting its utility is so far not clear[62].

Anemia associated with sirolimus may be due to several action mechanisms: interference with the proliferation of more primitive erythroid progenitors[63] which is more evident in combinations with cyclosporine[16], or certain resistance to the action of erythropoietin[64]. On the other hand, the microcytic anemia profile acquired without iron deficiency suggests altered iron metabolism, which could be grouped into two different entities: a) chronic disorder anemia, without having related sirolimus for now with either clinical or analytical evidence of inflammation (hepcidin, an acute phase protein considered to mediate in inflammation anemia, is not affected by sirolimus)[60]; or b) a drug-induced sideroblastic anemia that could respond to treatment with vitamin B6, without the presence of marrow ring sideroblasts having yet been reported.

**Table I.** Hemoglobin (g/L) in trials with sirolimus

| Trial | Time | All with cyclosporine and steroids | | | |
|---|---|---|---|---|---|
| | | SRL 2 mg/d | SRL 5 mg/d | Placebo | AZA |
| 301[1,2] | Month 1 | 101 | 101 | – | 100 |
| | Month 24 | 131 | 131 | – | 135 |
| 302[2,3] | Month 1 | 99 | 97 | 98 | – |
| | Month 24 | 134[a] | 133[a] | 142 | – |
| | **Time** | **All with steroids** | | | |
| | | **SRL + AZA** | **CsA + AZA** | | |
| 207 | Month 1 | 93[b] | 110 | | |
| | Month 24 | 137 | 135 | | |
| | | | | **SRL + MMF** | **CsA + MMF** |
| 210 | Month 1 | | | 99[c] | 108 |
| | Month 24 | | | 141 | 133 |

[a]p < 0.05 *versus* placebo; [b]p < 0.001; [c]p< 0.05.

### Recommendations

Several of those present at the meeting expressed that anemia was observed more frequently in conversions than in «de novo» uses and particularly in the aging population. Anemia was considered more clinically relevant than leukopenia or thrombocytopenia. Anemia associated with sirolimus after transplant is probably multifactorial and influenced by advanced renal failure situations and concomitant treatments such as angiotensin converting enzyme inhibitors or angiotensin II receptor or mofetil mycophenolate antagonists. With respect to the latter, withdrawal of cyclosporine may be followed by an increase the levels of mycophenolic acid (MPA)[65-69]. Therefore, it seems reasonable to limit the maximum

dose of MMF to 1 g/d, or therapeutic drug monitoring.

### Leukopenia and Thrombopenia

In sirolimus development trials, it was observed that although both the leukocyte and platelet figures tended to be statistically less in the groups with greater exposure to sirolimus and in the combinations with an antimetabolite, the values reached were quite far from being clinically significant. The following table shows the platelet figure in several trials, the leukocyte figure being very similar to this.

Hong and Kahan showed results of a retrospective study in which immunosuppressive cyclosporine plus steroid therapy was compared to sirolimus plus cyclosporine and steroids. They observed an increase of 2.2 times in the relative risk of thrombocytopenia (defined as less than 150 *109/L) in the SRL group. Thrombocytopenia was observed in 78% of the patients in the SRL group, occurring in 88% of the cases during the first 4 weeks of treatment. An 8 times higher relative risk of leukopenia (defined as less than 5 *109/L) was observed in the group with SRL. There was a correlation between the occurrence of thrombopenia or leukopenia and the existence of levels > 16 ng/mL by HPLC[70] No pre-transplant variable associated with the risk of thrombopenia was found. Even more interesting was the analysis of the evolution of the leukopenia and thrombopenia: 87% of the thrombopenia cases and 91% of the leukopenia cases spontaneously improved with no intervention. In remaining cases a sirolimus dose reduction or temporary withdrawal of the drug were enough to resolve the episode. No patient needed to definitively suspend sirolimus due to leukopenia and thrombopenia.

The mechanism of the leukopenia origin may be based on a lack of response to different hematopoietic cytokines observed in vitro[71].

**Table II.** Platelets (*109/L) in trials with sirolimus

| Trial | Time | All with cyclosporine and steroids | | | |
|---|---|---|---|---|---|
| | | SRL 2 mg/d | SRL 5 mg/d | Placebo | AZA |
| 301[1,2] | Month 1 | 196[a] | 186[a] | – | 208 |
| | Month 24 | 222 | 227 | – | 225 |
| 302[2,3] | Month 1 | 198 | 191 | 199 | – |
| | Month 24 | 236 | 215 | 226 | – |
| | **Time** | **All with steroids** | | | |
| | | **SRL + AZA** | **CsA + AZA** | | |
| 207 | Month 1[b] | 161 | 261 | | |
| | Month 24[c] | 202 | 262 | | |
| | | | | **SRL + MMF** | **CsA + MMF** |
| 210 | Month 1[b] | | | 182 | 233 |
| | Month 24[d] | | | 173 | 213 |

[a]p < 0.05 compared to placebo and 2 mg; [b]p < 0.001; [c]< 0.05; [d]p < 0.01.

## THROMBOTIC MICROANGIOPATHY

The USRDS registry recently analyzed the development of thrombotic microangiopathy (TMA) (hemolytic uremic syndrome / thrombotic thrombocytopenic purpura) in a historical cohort of 15,870 renal transplant recipients between January-98 and July-00 (72). The following risk factors for de novo HUS were found in the multivariate analysis: recipient age (< 35 years versus >57), Hazard ratio 5.8; donor age (>48 years versus <24), HR 3.1; sirolimus upon hospital release, HR 2.6; male recipient, HR 0.5. The absence of significance of the use of sirolimus in induction together with the inability to trace the causes of the immunosuppression changes made the authors request precaution when assessing these results which could reflect that sirolimus was present upon hospital release as a result of salvage therapy in patients with TMA.

### SRL in Concomitant Treatments With Calcineurin Inhibitors

In Phase III studies in which all patients were taking cyclosporine and prednisone, several cases of hemolytic uremic syndrome (HUS) were included which are specified in the table below:

An increased HUS rate was observed in study 302. HUS/TTP was diagnosed in 6 of the 18 randomized patients in a single center. The HUS/TTP cases were generally reversible with suspension of CsA, SRL or both.

The University of Texas group performed a retrospective review of their transplanted patients with the CsA, SRL and steroid combination[73]. 10 (1.5%) of the 672 patients treated with this com-

bination developed HUS. 7 of these 10 patients showed other concomitant adverse effects, such as acute rejection or infections (herpes simplex and pancolitis). Glomerulonephritis was the cause of terminal kidney disease in 7 of the 10 cases. The drug concentration mean was 294 ng/mL CsA and 20 ng/mL sirolimus, slightly exceeding their target levels. Treatment of these patients included withdrawal of sirolimus (it was subsequently re-introduced in three patients) or cyclosporine (re-introduced in six). 9 of the 10 kidneys were functioning normally 24 weeks after diagnosis (mean serum Cr 1.6 +/- 0.59 mg/dl). The tenth patient received a transplant removal and died due to refractory thrombopenia, Aspergillus infection and multiorgan failure.

Other cases of thrombotic microangiopathy have been reported in concomitant treatments with calcineurin inhibitors[74-76]. Sirolimus was suspended in some of these cases[74-76] and the calcineurin inhibitor was suspended in other cases[74]. In hematopoietic progenitor transplants, the Dana Farber group suggests that the sirolimus and tacrolimus combination achieves low graft versus host disease and mucositis rates, but at the expense of a greater frequency of a type of hemolytic uremic syndrome with a better prognosis in its historical series with cyclosporine[77]. It has also been suggested that the hemolytic uremic syndrome would be more frequent in the combined treatments of sirolimus with cyclosporine than with tacrolimus, perhaps because this combination would induce greater endothelial cell necrosis[78].

### SRL in concomitant treatments without calcineurin inhibitors

Eight cases of thrombotic microangiopathy have also been reported in calcineurin inhibitor-free regimens[79-83], although the cause of chronic renal disease that provided the grounds for the transplant in two of these cases was non-epidemic primary hemolytic uremic syndrome[80]. In some of these cases, HUS would be associated to a decrease in intrarrenal expression of vascular endothelial growth factor (VEGF)[82].

**Table III.** HUS rate (5) in studies 301 and 302

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| % (n) | SRL 2 mg/d | SRL 5 mg/d | Placebo | AZA |
| 301 and 302 combined | 2.0 (10/502) | 5.6* (26/482) | 1.6 (2/124) | 1.8 (3/161) |
| 301 | 1.4 (4/284) | 4.0 (11/274) | N/A | 1.8 (3/161) |
| 302 | 2.7 (6/218) | 7.6**(16/208) | 1.6 (2/124) | N/A |

*P < 0.05 SRL 5 mg/d *vs* SRL 2 mg/d.
**P < 0.05 Fisher exact test between groups.

### SRL calcineurin inhibitor salvage treatments

On the other hand, the successful use of sirolimus in converting transplant recipients with a history of HUS due to calcineurin inhibitors has been reported[27,84-91].

Franco et al. communicated the use of sirolimus in 10 patients with «de novo» HUS evidenced by biopsy. After abruptly withdrawing the calcineurin inhibitor, a significant improvement in renal function was observed one month after the transplant. When the mean 19-month follow-up concluded, 8 of the 10 patients maintained the graft, another one of the patients was undergoing hemodialysis and the other one had died due to sepsis shortly after beginning with sirolimus[86]. At the University of Tennessee, 12 cases of fast conversion to sirolimus due to HUS were reported, adding daclizumab to kidney and pancreas transplant patients. This was resolved in all cases with histological confirmation in the five biopsied cases[27].

It is still not clear if sirolimus may be useful for those patients whose primary cause for transplantation is an HUS[80,91].

## INFECTIONS

### Viral Infections

*Herpes*

A higher number of mucosal lesions presumably due to herpes simplex in patients treated with higher doses of sirolimus than in the azathioprine[1,2] or placebo[2,3] arms have been reported. Even higher doses of sirolimus (levels up to 30 ng/mL) were used in a study that compared the efficacy of sirolimus to cyclosporine, both being combined with azathioprine[92]. Mucositis due to herpes simplex was reported in 10 (24%) patients with sirolimus, compared with 4 (10%) patients with CsA. Most of these diagnoses were made based on clinical and non-microbiological criteria.

No clinically significant interactions with acyclovir have been observed[49].

*Varicella-zoster*

In study 310 more episodes of herpes zoster were reported in the treatment group combined with cyclosporine, than in the arm in which the latter was suspended[35,93].

*Cytomegalovirus*

Dana Farber's experience in marrow transplant suggests that the cytomegalovirus incidence is low[94], and different groups have reported similar findings in de novo heart transplant[95], liver transplant[96] or renal transplant[57,97,98]. Furthermore, one of the largest renal transplant series converted to sirolimus found no episodes of reactivation after conversion[99]. Similar beneficial findings with regard to CMV infection have been reported with other mTOR inhibitors such as everolimus in both heart[100] and renal transplantation[101]. These results have led to successfully attempting coadyuvant sirolimus treatment with ganciclovir in cases of CMV infections that are resistant to the latter[102].

*Herpes virus 6*

It has been reported that seroconversions to herpes virus 6 are greater in regimens with sirolimus or daclizumab than in all others[103].

*Epstein-Barr Virus*

Sirolimus controls in vitro the growth of lymphomatous lines transformed by the Epstein Barr virus (EBV)[104]. In vivo, it seems that no reactivations have been reported in broad series conversions[99].

In adults treated with sirolimus, cyclosporine and steroids at the University of Texas, the incidence of post-transplant lymphoproliferative disease seemed to be quite less than that found with the tacrolimus plus mycophenolate combination[97]. However, a recent trial on a pediatric population that used daclizumab, calcineurin inhibitors, sirolimus and steroids, and that after 6 months randomized to withdraw the steroids or not, had to be suspended since there was a high incidence of PTLD in younger EBV-negative patients[105].

*Human Immunodeficiency Virus*

From a preclinical point of view, sirolimus at very low doses decreases the CCR5 chemokine expression necessary for the human immunodeficiency virus (HIV) to enter T lymphocytes[106]. It further inhibits HIV-induced apoptosis in CD4 lymphocyte syncytium[107,108].

From the clinical point of view maintenance immunosuppression with cyclosporine, sirolimus and steroids, after induction with basiliximab, together with highly effective antiretroviral therapy achieved good virological control with no opportunistic infections in 40 HIV-positive kidney recipients[109].

From the pharmacokinetic point of view sirolimus, similarly to other immunosuppressants, would have interactions with protease inhibitors[48,49,109-111].

*Hepatitis Virus*

Preclinical data on the role of sirolimus as a hepatic antifibrotic agent[112,113] suggests that it could play a role in transplants in hepatitis B (HBV) or C (HCV) virus-positive recipients. However, available preclinical data on the interaction of sirolimus with HBV or HCV are very limited. The Denver group has reported their experience in liver transplants in HCV-positive patients who were given sirolimus as a baseline therapy without finding any differences in survival with respect to their previous immunosuppression schemes[114]. Two anecdotic cases of patients who attained virologic control of HCV after switching to sirolimus in liver transplantation have been recently reported[115]. There is no data regarding hepatitis B.

*BK Virus*

There is very little and contradictory data: In de novo transplants, the Texas group has reported a very low incidence of infection[97]. The successful conversion to sirolimus has been reported in four cases thus controlling nephritis[116,117], another case of unsuccessful conversion[90], and three other cases in which nephritis struck during treatment with a combination of sirolimus, mycophenolate and induction with antibodies[118].

**Fungal Infections**

Sirolimus has in vitro antifungal activity[119,120], in fact it was initially identified as an anti – Candida albicans antifungal antibiotic. It has anti- Cryptococcus neoformans, Candida albicans, and Aspergillus fumigatus activity. It is synergistic with cas-

pofungin against Aspergillus species[121]. Sirolimus pharmacokinetically interacts with azoles, given that the latter tend to increase sirolimus AUC[48,49,122-124].

In the first conversion experiments several cases of pneumonia due to Pneumocystis jiroveci (previously carinii) in patients who had not performed prophylaxis were reported[125-127].

### Bacterial Infections

Trials of combinations of sirolimus plus cyclosporine did not show in general more infections with sirolimus than in the control groups[2]. Both respiratory and urinary bacterial infections have been reported with sirolimus[97,128], although a study randomized between the tacrolimus-sirolimus combination compared to the tacrolimus–mycophenolate combination showed no differences in terms of bacterial infections after 6 months[129]. The occurrence of bacterial infections may be due to the capacity of sirolimus to inhibit neutrophil migration[130], as well as the inhibition of IL-10 production by mononuclear cells[131].

### Recommendations

Most of those present agreed that it is not necessary to use any type of extraordinary infectious prophylaxis when using sirolimus. Prophylaxis against Pneumocystis jiroveci (previously carinii) during the first year of the transplant is currently recommended in the summary of product characteristics[49], and trimethoprim-sulphamethoxazole does not seem to affect the pharmacokinetics of sirolimus[132].

Even though the data concerning the role of sirolimus on CMV is promising, for the time being the prophylaxis policy must be similar to that performed with calcineurin inhibitors or mycophenolate mofetil.

## EDEMAS

Given the difficulties in quantization, the frequent prevalence of edemas in the renal transplanted population and the likely under-reporting thereof, there is a great heterogeneity in the reported incidence of edemas in trials with sirolimus. In this context, it is specially useful the blind design of trials such as the combinations of sirolimus plus cyclosporine that show that approximately half of the patients refer edemas at 2 years without differences with the control groups of azathioprine and /or placebo[2].

The Necker Hospital group thoroughly reviewed skin disorders occurring during treatment with sirolimus by means of a transverse study that dermatologically evaluated all patients who had been receiving sirolimus from March to June of 2003[133]. Chronic edemas (defined as lasting for more than 1 month, resistant to diuretics and with no underlying local, renal or cardiac causes) were found in 55% of the patients, and angioedemas (defined as acute subcutaneous edemas resolved in less than 4 days) were found in 15% of the cases. Most of the chronic edemas were located in lower members, were soft with no inflammation, and a traumatic episode was described in several cases as the triggering event. Some other episodes were described in upper members or eyelids. The cases of angioedema developed a few hours after beginning with sirolimus and except in one case, they disappeared between 1 and 4 days with no treatment. The angioedema was located on the face and in the oral cavity in most cases, and in two-thirds of the cases, participation of concomitant treatment with angiotensin converting enzyme inhibitors (ACE inhibitors) or angiotensin II receptor blockers could not be ruled out in the symptoms. The interaction of these drugs with sirolimus has also been suggested by the description of seven cases of angioedema of the tongue or face with the combined administration of sirolimus with ramipril or enalapril. In these cases the edema disappeared with the decreased dose of both drugs, or withdrawal of ACEI or of both[134,135].

Other authors have also described isolated cases of edema in atypical locations, such as on the eyelids or tongue with either sirolimus or everolimus[134,136-140], or typical peripheral edemas in lower limbs after conversion[141,142].

As with other adverse effects associated with sirolimus, edemas also seem to be more prevalent when sirolimus is used in combinations with calcineurin inhibitors (see table IV).

The mechanisms underlying sirolimus-induced edemas are currently unknown. It does not seem to be originated in a net positive balance of water and sodium, as patients on sirolimus exhibit usually a lower increase in weight than with other immunosuppressive agents, and has been described in patients with good renal function and without apparently relationship with the additionally described sirolimus associated proteinuria. Hypothetical mechanisms could be alterations in the expression of VEGF or PDGF.

**Table IV.** % of edema reported as an adverse event in different trials

| Trial 310[35-37,93] | CsA+SRL+Ste | SRL+Ste | P |
|---|---|---|---|
| After 1 year | Not available | Not available | |
| After 2 years | 8.4 | 2.8 | 0.01 |
| After 3 years | 10.2 | 4.2 | < 0.05 |
| After 4 years | 10.7 | 4.2 | < 0.05 |
| After 5 years | 10.7 | 5.1 | < 0.05 |
| Trial 212[143] | CsA+SRL+Ste | SRL+Ste | |
| After 1 year | 14.4 | 3 | 0.005 |
| Trial 100520[38] | High TAC+SRL | Low-TAC and Stop + SRL | |
| After 1 year | 42 | 29 | NS |

### Recommendations

Although there is no clear cause as to the origin of edemas associated with sirolimus, different measures were suggested to minimize them (table XVI), even when there is no published experience to that effect.

Low doses of diuretics may be used although success is modest and could eventually worsen renal function.

## RENAL FUNCTION

When the effect of sirolimus on renal function was initially studied, it was observed that it did not exhibit nephrotoxicity associated with cyclosporine, both in animal models[144] and in clinical trials on patients with psoriasis[145]. However, sirolimus combinations with cyclosporine seemed to exhibit greater nephrotoxicity than the latter drug alone[1,3,146]. Podder et al. confirmed the existence of this nephrotoxic synergy in rats and attributed it to an increased intrarenal cyclosporine concentration[16]. In this sense, trials that have compared therapy with sirolimus after suspending calcineurin inhibitors have found better renal function compared to the combined maintenance of these drugs with sirolimus[35-39,52,93]. It seems in murine models that the combination of sirolimus with cyclosporine is more nephrotoxic than with tacrolimus[147]. This same finding has been repeated in several clinical experiments in liver transplants[148,149] or kidney transplants[51], although other authors have not found this pattern[150].

The available information regarding whether the maintained combination of tacrolimus with sirolimus is more nephrotoxic than the more standard combination with mycophenolate is more scarce due to the existence of studies with less recruitment, but there is more work that suggests this[95,29,151-153], than work that suggests the opposite[54,154].

On the other hand, potassium serum figures in treatment with sirolimus tend to be lower than when they are compared with azathioprine, placebo or cyclosporine, there being good response to potassium supplements[92,155,156]. Contrary to what occurs with cyclosporine, sirolimus seems not to affect uric acid or magnesium serum levels[156]. The results of other serum analytical parameters in relation to renal tubular function are detailed in table XIV

### Proteinuria

*De novo experiments*

The first animal experiments that evaluated renal function did not analyze proteinuria. Recently, Bonegio et al. have shown that sirolimus decreases inflammation and fibrosis in an experimental murine model of membranous nephritis with proteinuria[157], and proteinuria decreases in NZB/WF1 models with lupus[158,159].

Unfortunately, proteinuria was not included as a quantitative parameter in overall sirolimus development trials with a large number of recruited patients either. However, the incidence reported as an adverse event was similar in the arms with sirolimus and with the control groups. In the study that tested cyclosporine elimination 3 months after the transplant, an intention to treat analysis showed improvement in renal function after 5 years, even in patients in whom post-randomization proteinuria had been reported[160]. Other «de novo» randomized studies with less recruitment have reported proteinuria after transplant and their results are expressed in table V below.

*Conversion Experiments*

Morelon et al, retrospectively disclosed several cases of proteinuria in patients with renal transplant who were converted to SRL. Thirty-two of the 50 patients exhibited proteinuria, 18 of them had proteinuria within the nephrotic syndrome range. None exhibited focal segmental glomerulosclerosis lesions in the biopsy before changing to SRL. Focal segmental glomerulosclerosis was shown in 5 of the 15 biopsy cases, although none exhibited lesions in the biopsy before changing to SRL. Proteinuria signifi-

**Table V.** Proteinuria in g/day in «de novo» randomized trials

| Author | Arms | N | 6 months | 1 year | 2 years |
|---|---|---|---|---|---|
| Flechner[161] | Bas + SRL + MMF + Ste | 31 | ND | ND | 0.55 |
| | Bas + CsA + MMF + Ste | 30 | ND | ND | 0.88 |
| Morales[162] | TAC withdrawn + SRL | 44 | ND | ND | 0.3 |
| | TAC + SRL | 43 | ND | ND | 0.5 |
| Glotz[163] | Thy + SRL + MMF + Ste | 71 | 0.34 | ND | ND |
| | TAC + MMF + Ste | 70 | 0.82 | ND | ND |
| Lebranchu[164] | ATG + SRL + MMF + Ste | 71 | ND | 0.64[a] | ND |
| | ATG + CsA + MMF + Ste | 74 | ND | 0.18 | ND |
| Hamdy[56] | BAS + SRL + TAC + Ste | 65 | 0.66 | 0.52 | ND |
| | BAS+ SRL + MMF + Ste | 67 | 0.90 | 1.01 | ND |

p = NS in all comparisons.

cantly decreased in 6 patients after starting treatment with ACEIs[165]. Later, Letavernier et al. retrospectively analyzed the combined experiment in converting to sirolimus in two hospitals in Paris. The experience of 68 patients was recorded. Starting from 0.4 g/day baseline proteinuria, they observed an increase at months 6, 12 and 24. Proteinuria improved in the 19-patient subgroup in whom sirolimus treatment was suspended and treatment with CNI was re-established[166].

The Strassburg group reported the results of a retrospective observational study that examined proteinuria development in 59 liver (n=30) or kidney (n=29) transplant patients after starting treatment with SRL. Only two liver transplant patients had proteinuria, whereas baseline proteinuria increased in 14 of the 29 kidney transplant patients. A significant correlation was observed in this last patient subgroup between the proteinuria increase and CsA or tacrolimus concentration decrease. No correlation was found with sirolimus levels[167]. These data of a lower reported proteinuria in native kidneys could suggest that this proteinuria may be linked to a preexisting impairment associated with chronic allograft nephropathy. Nevertheless, episodes on a increase in proteinuria have been reported in heart or pancreatic islet recipients[168,169].

A very few histological data in sirolimus-associated proteinuria have been described[170-172], and almost never a previous baseline biopsy was available befote conversion. Non-specific cases compatibles with IgA nephropathy, membranous or membranoproliferative glomerulonephritis, focal and segmental hyalinosis or double contour glomerulous have been found.

Dittrich et al. have described four patients in whom proteinuria and renal function impairment were observed after converting to sirolimus and who exhibited data compatible with glomerulonephritis in the biopsy: two cases of IgA, one case of membranous GN and another case of proliferative membranous GN. After re-introducing the calcineurin inhibitors the proteinuria remitted and the glomerulonephritis data disappeared in two cases in which a second biopsy was performed[170].

In pediatric renal transplants, Butani et al. have recently communicated their experience with 13 children converted to sirolimus. An increased urine protein / creatinine ratio was observed in 12 of them. Complete nephrotic syndrome with anasarca and hypoalbuminemia of 1.5 g/dl was developed in one of these 12 cases, and the biopsy showed chronic nephropathy data, whereas the serum albumin figure was not affected in the rest. All the patients could be maintained with sirolimus by means of different maneuvers including the use of angiotensin receptor antagonists. A non significant tendency was found between proteinuria and complete withdrawal of the calcineurin inhibitor[173].

Diekmann et al. analyzed their conversion experience. They found that the main predictive factor for improvement in renal function was a baseline proteinuria of less than 800 mg/d, and not the baseline glomerular filtrate[61]. Preliminary results from randomized 316 CONVERT trial seem to show that statistically distribution of proteinuria does not follow a normal shape, with extreme high values in a little set of patients, and higher increments in proteinuria in those patients with higher baseline values. Glomerular filtration rate would improve in patients with baseline urine protein /creatinine ratio lower than 0.5.

Saurina et al. have suggested the existence of a hemodynamic effect upon withdrawing the calcineurin inhibitors, which would make renal blood flow and intraglomerular pressure increase and renal function reserve decrease, establishing a state of hyperfiltration[174]. Other proposed possible mechanisms are a tubular damage that would eventually reduced protein absorption[175], or an alteration in the expression of vascular-endothelial growth factor[176], or the inhibition in the repair mechanisms of endothelial glomerular cells[177].

In conclusion, the role of baseline proteinuria as a predictor of outcomes after transplantation is progressively growing[61,172]. The experiences that have not excluded patients taking into account the baseline proteinuria have reported increments in proteinuria value approximately in 30% of patients[171,172], and the management have included the successful use of ACEi or ARB-III[171,172], or the restart of calcineurin inhibitors.

*Experiences outside the transplant*

Incidental clinical experiences have been communicated in patients with glomerulonephritis with both negative[178] and positive[179,180] results.

## Delayed Graft Function

None of the four big trials with sirolimus included delayed graft function as a mandatory variable in the study. The percentages in table VI below are in some cases the percentage of patients dialyzed during the first week after the transplant and in others the percentage of cases in which acute tubular necrosis was reported as an adverse event. Since the criteria for post-transplant dialysis are not standardized and the possible bias in reporting adverse events, these percentages should be considered for guidance only.

Given the different adverse event profile of sirolimus from that of the calcineurin inhibitors, one of the first groups of patients thought of as beneficiaries of the use of sirolimus were the patients with a risk of DGF or with established DGF:

- Thus, the University of Texas group communicated its experience in patients with DGF risk: deceased donors under 10 or over 60 years old, death by vascular accident, or hypotension or perioperative oliguria. It examined the benefits of extending the introduction time of cyclosporine by means of administering a regimen with basiliximab, sirolimus and corticoids[181]. The same Texas group has more recently communicated that their strategy for DGF risk patients is the use of sirolimus with delayed introduction of cyclosporine and induction with basiliximab for low-risk patients and thymoglobulin for high-risk patients[182]. Cyclosporine is introduced in a mean of 12 days. They obtained an acute rejection rate of 10% and 3%, and DGF (dialysis in the first week) of 12% and 7% in low and high risk groups, respectively, with this approach.
- A similar scheme was followed by Chang et al., except for their use of daclizumab in the induction in 14 renal receptors[183] and incorporating mycophenolate in the maintenance. Two patients (14%) showed acute rejection and post-transplant dialysis was needed in nine patients. Mean creatinine changed from 8.4 mg/dl at the starting time of sirolimus to 2.1 mg/dl one month after the transplant.
- Pescovitz et al. used a combination of sirolimus and mycophenolate without antibodies in nine patients with DGF[184].
- Mital et al. used an induction with thymoglobulin followed by sirolimus, mycophenolate and a quick withdrawal of steroids in 23 patients without immediate renal function[185]. Two patients (8%) showed acute rejection and mean creatinine at the end of the follow-up period (up to one year) was 1.5 mg/dl.
- El-sabrout et al. used a combination of sirolimus with mycophenolate preceded by induction with basiliximab or ATG[186]. Overall 71% of the patients suffered DGF with a mean duration of 17 days of dialysis.
- In Nashville University, Shaffer et al. treated 19 patients with DGF or suboptimal donors who were induced with thymoglobulin or basiliximab followed by maintenance with sirolimus, MMF and prednisone. The DGF rate was not reported and there was 16% acute rejection. Mean creatinine in the last follow-up was 1.6 mg/dl[187].
- Vincenti et al. tested the combination of daclizumab, sirolimus, mycophenolate, steroids and delayed introduction of cyclosporine at a median of 26 days after the transplant followed by sirolimus or mycophenolate withdrawal in 30 patients with DGF. This protocol achieved acceptable rates of acute rejection only in the

**Table VI.** DGF in several trials with sirolimus

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| **Study** | **SRL 2 mg/d** | **SRL 5 mg/d** | **Placebo** | **AZA** |
| 301 | 36 (12.7) | 35 (12.8) | – | 28 (17.4) |
| 302 | 40 (17.6) | 44 (20.1) | 27 (20.8) | – |

p = NS in all comparisons.

| | All with steroids | | | |
|---|---|---|---|---|
| | **SRL + AZA** | **CsA + AZA** | | |
| 207[a] [92] | 7 (17.1) | 3 (7.1) | | |
| | | | **SRL + MMF** | **CsA + MMF** |
| 210[b] [155] | | | 10 (25) | 9 (23.7) |

[a]% acute tubular necrosis, defined as «the need for post-transplant dialysis», p = N.S.

[b]% DGF, defined as «dialysis in the 1st week», p = N.S. The mean duration of the dialysis was 4.1 and 16.8 days in the CsA and sirolimus groups, respectively, p = 0.03.

non-Afro-American population (23% versus 62%)[188].

In short, most of these first published experiments were not controlled, had a scarce number of patients and were mostly based on delaying introduction of the calcineurin inhibitor. Although graft survival and renal functions were generally very acceptable, heterogeneity in all these cases and in the short follow-up time, limit the conclusions on the role of sirolimus in DGF.

The origin of the controversy on the role of sirolimus in DGF appears with two studies on animals carried out by Lieberthal et al. In the first of these, in vitro, they concluded that sirolimus inhibits proliferation and induces tubular cell apoptosis. In the second, in vivo, they showed that sirolimus delayed improvement in renal function after an ischemic injury[189]. Several retrospective reviews have subsequently tried to analyze this controversy:

- McTaggart et al. communicated their series of 132 patients with DGF (dialysis within the first week) of a total of 563 (23%) transplant patients from January 1997 until June 2001. Very predictive factors for DGF development such as donor age and cold ischemia time were not predictive for DGF duration. However, exposure to sirolimus was very predictive of DGF duration (RR 0.48; 95% IC 0.3-0.7, p = 0.0007)[190]. Evolution one year after the transplant in the 132 cases with DGF was similar regarding graft survival and renal function, independently from the immunosuppression received with sirolimus or not[191].
- Smith et al. reviewed the experience of living and deceased donor renal transplants carried out in Washington University treated with several immunosuppressant schemes. They found that the incidence of DGF (defined as dialysis beyond the first 24 hours of the transplant) was more frequent in patients treated with sirolimus (25% versus 8.9%, P = 0.02) and correlated with sirolimus dose. Multivariate analysis showed that DGF development was related to sirolimus use and protecting factors were living donor and PRA exceeding 0% (this last finding probably related to a higher usage of thymoglobulin and delayed CNI introduction). Regarding DGF mean duration in the multivariate analysis this was positively related to donor age and negatively related to MMF dose, whereas sirolimus use lost significance. All the biopsies performed during the DGF episodes showed tubular damage. Furthermore, amongst the 22 biopsies performed in DGF and treatment with sirolimus status, nephropathy data due to cylinders was found for 12 patients[192].
- The Tennessee group evaluated their series of deceased donor renal transplant patients using several definitions of DGF. Sirolimus use with calcineurin inhibitors was associated with the need of dialysis during the first week, and with creatinine non-improvement in the first 3 days, but not with creatinine greater than 3 mg/dl on the 5th day. These associations were not found when sirolimus was associated with mycophenolate. Renal function a year after the transplant in DGF patients was similar whether treated with sirolimus or not[193].
- Previous experiments analyzed the joint use of sirolimus and calcineurin inhibitors. The Cleveland group has not found differences regarding DGF rates (dialysis in the first week), nor slow renal function (creatinine greater than 3 mg/dl on day 5 without dialysis) between their cohort of transplant patients with sirolimus without calcineurin inhibitors (n=287) and their cohort with calcineurin inhibitors (n=282). Factors independently related to DGF were deceased donor, diabetes mellitus as cause of terminal renal failure and male sex[194].

Two randomized post-marketing studies have communicated their DGF rates in the arms with and without sirolimus. Stallone et al. carried out a trial in suboptimal kidney recipients between two groups, one with sirolimus plus CsA at low doses with subsequent withdrawal (n=42), and the other with full doses of CsA and MMF (n=48). All the patients received induction with basiliximab and corticoids. DGF incidence was similar in both groups, whereas DGF duration was greater in the sirolimus group (19 days versus 10 days). However, there were no differences in patient survival, graft survival or renal function between both groups after one year[195]. Another trial has compared two treatment schemes based on tacrolimus and steroids with sirolimus or mycophenolate. The DGF rate (dialysis in the first week) was 22% and 31%, with sirolimus and mycophenolate respectively[129].

**Recommendations**

The best way of obtaining performance of sirolimus as a renal function preserver is by means of calcineurin inhibitor-free schemes[11,161,187, 196-198] or

by means of short treatments combined with these and subsequent suspension of the calcineurin inhibitor 2-3 months after the transplant. In this last approach it is probably preferable to associate sirolimus with tacrolimus instead of cyclosporine in almost all cases except in hepatitis C virus-positive patients due to the risk they have of post-transplant diabetes.

Until the role of sirolimus in post-transplant proteinuria is clarified, it seems reasonable to maximize precautions when converting patients with a proteinuria greater than 1 gram daily. The use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers could improve proteinuria associated with sirolimus although there is not sufficient evidence to recommend an anticipated treatment with them. The alternative of combining sirolimus with low doses of calcineurin inhibitors must be balanced with the risk of increasing the nephrotoxicity of the latter[16].

Regarding DGF, most of those present in the meeting thought that sirolimus did not increase the incidence of DGF, although it could possibly increase the duration. They agreed in that having overcome DGF there are no reasons to contraindicate the long-term use of sirolimus. In future prospective studies with sirolimus it will be necessary to include a complete evaluation of DGF, including the percentage of dialysis use in the first week, the time until reaching creatinine under 3 mg/dl and the time until the last dialysis as study variables. The use of delayed sirolimus introduction protocols with introduction delayed to 5-7 days could be useful in minimizing these problems.

## THROMBOSIS

From the preclinical point of view the Regensburg University group has found that as well as having an antiangiogenic effect, sirolimus favors thrombosis development inside tumors, not affecting the peritumoral tissue[199].

The current controversy on the role of sirolimus and thromboses derives almost exclusively from liver transplant trials using de novo sirolimus and the observed percentage of hepatic artery thrombosis between the experimental and control arms (see table VII).

It is advisable to put these figures in perspective. Some retrospective series of patients treated with different immunosuppressive have found percentages of hepatic artery thrombosis between 2% and 7%[201-203], with several factors involved that include age, disparity in age, weight or sex between donor and recipient, the role of cytomegalovirus or surgical techniques variants.

**Tabla VII.** «De Novo» liver transplant trials

| | TAH % |
|---|---|
| Trial 220 (n = 222): | |
| SRL + TAC + Ste* | 6/110 (5.5%) |
| *vs* | |
| TAC+Ste | 1/112 (0.9%) |
| Ensayo 211 (n = 163) (200 (p = NS): | |
| SRL + CsA + Ste | 14/111 (12.6%) |
| *vs* | |
| TAC + Ste | 2/52 (3.8%) |

*: all in the first 16 days.

However, other retrospective experiments in de novo liver transplant have not found this association between sirolimus and thrombosis[128,204].

In the two great sirolimus, cyclosporine and steroid combination in liver transplant trials, the groups with sirolimus did not show a different incidence of graft losses due to the renal artery or vein thrombosis that the control groups with azathioprine or placebo showed (global incidence of 1,1% in both trials)[1-3]. The University of Texas group carried out a retrospective review of their cohort of patients treated with sirolimus and cyclosporine and compared it to another cohort treated with cyclosporine and azathioprine without finding differences between them regarding thrombosis incidence (in lower limbs, kidneys or lungs)[205]. They found an association between thrombotic episodes of the sirolimus group and the previous development of ipsi- or contralateral lymphoceles.

### Recommendations

Although the association between sirolimus and thrombosis is very far from being obvious, it seems reasonable not to recommend its use immediately after liver transplant and is thus suggested in the current summary of product characteristics (48, 49). Differential diagnosis of thrombotic episodes after solid organ transplant includes detecting acquired situations such as the occurrence of anticardiolipin antibodies[206-208], or in cases of liver transplants with donor transmitted heterozygote forms of thrombophilia such as S protein deficiency[209], C protein deficiency[210], factor V Leiden deficiency[211-221] or prothrombin gene mutations[222]. When studying these acquired thrombophilias

analytical discrepancies may be found that could be demonstrated, for example, by means of positive recipient activated C protein phenotypic resistance tests, associated to factor V Leiden genetic analyses that are negative in the recipient's blood and positive in the grafted liver (or the donor's blood if available)[223].

It does not seem to be necessary to establish any special measures regarding renal transplant.

## APHTHAES

A greater number of mucosal lesions presumed to be due to herpes simplex has been communicated in patients treated with higher doses of sirolimus than in arms with azathioprine[1] or placebo[3]. Even higher sirolimus doses (levels up to 30 ng/ml) were used in a study comparing the efficacy of sirolimus to cyclosporine, both combined with azathioprine[92]. Mucositis due to herpes simplex was reported in 10 (24%) of the patients with sirolimus, compared to 4 (10%) of the patients with CsA. Most of these diagnoses were made by clinical criteria and non-microbiological criteria.

The summary of the incidence of adverse effects reported in sirolimus studies is shown in table VIII below:

Van Gelder et al. carried out a multicenter randomized prospective study in 33 kidney transplant recipients maintained without steroids and with tacrolimus and mycophenolate[224]. They were randomized to continue with TAC and MMF (n=18) or to suspend tacrolimus and start with sirolimus, seeking levels between 10-15 ng/ml (n=15). The study had to be prematurely suspended due to the appearance of oral ulcers in nine patients. The injuries disappeared in two weeks after withdrawing sirolimus. The herpes virus was not cultured in any of the patients. These authors suggested that part of these aphthaes would improve after administering tablets instead of the oral solution[224,225]. However, Wyeth study 309, which randomized to receive oral solution against tablets, along with cyclosporine and steroids, did not find differences in the incidence of oral alterations in 1 year[226]

**Table VIII. Adverse effects communicated in trials with sirolimus**

| | Any sirolimus n = 4615, n (%) | Placebo n = 238, n (%) | Comparator n = 719, n (%) |
|---|---|---|---|
| Oral ulcers | 164 (4) | 7 (3) | 5 (< 1) |
| Gingivitis | 86 (2) | 2 (< 1) | 12 (2) |
| Glossitis | 66 (2) | 5 (2) | 6 (< 1) |
| Stomatitis | 143 (4) | 3 (1) | 11 (2) |
| Herpes simplex | 422 (10) | 12 (5) | 34 (5) |

### Recommendations

In order to test the possible usefulness of testing antiviral drugs it would be useful to know whether the etiology of these injuries is herpetic or not. At the moment, reducing the doses or temporary withdrawing the drug could be tried in order to minimize these effects. Symptomatic treatment with analgesics or rinses with local anesthesia such as lidocaine could improve these symptoms. The Necker hospital group has successfully used rinses with sucralfate[165].

## SURGICAL PROBLEMS: LYMPHOCELE, WOUND HEALING

### Lymphocele

The trials combining sirolimus with cyclosporine found a greater frequency of lymphoceles in the groups with sirolimus than in control groups (see table IX).

Two retrospective studies have analyzed respectively the combinations of sirolimus with cyclosporine or with tacrolimus, finding that in these combi-

**Tabla IX.** % of lymphoceles reported in trials with sirolimus

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| **Studies** | **SRL 2 mg/d** | **SRL 5 mg/d** | **AZA** | **Placebo** |
| 301[a] | 13.7% | 18.6% | 5% | |
| 302[b] | 12% | 15% | | 6% |

| | All with steroids | | | |
|---|---|---|---|---|
| | **SRL + AZA** | **CsA + AZA** | **SRL + MMF** | **CsA + MMF** |
| 207[c] | 12.2% | 11.9% | | |
| 210[d] | | | 7.5% | 0% |

[a]$p < 0.001$; [b]$p = 0.02$; [c,d]NS.

nations the incidence of lymphoceles is greater than in schemes without sirolimus[227,228].

### Surgical Wound Alterations

Due to the antifibrotic effect demonstrated in preclinical models of cirrhosis of the liver or bronchiolitis obliterans[112,229,230], the preoccupation developed that sirolimus could be associated with a greater rate of surgical problems. In the development trials a statistically non-significant trend towards a greater rate of surgical wound healing problems was shown in the arms with sirolimus with similar infection rates (see tables X and XI).

**Table X.** % anomalous healing reported in trials with sirolimus

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| **Studies** | **SRL 2 mg/d** | **SRL 5 mg/d** | **AZA** | **Placebo** |
| 301[a] | 9 | 10 | 5 | |
| 302[b] | 7 | 13 | | 6 |
| p = N.S. | | | | |

| | All with steroids | | | |
|---|---|---|---|---|
| | **SRL + AZA** | **CsA + AZA** | **SRL + MMF** | **CsA + MMF** |
| 207[c] | 7,3 | 2,4 | | |
| 210[d] | | | 2,5 | 0 |

a, b, c, d: p = N.S.

**Table XI.** % surgical wound infection reported in trials with sirolimus

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| **Studies** | **SRL 2 mg/d** | **SRL 5 mg/d** | **AZA** | **Placebo** |
| 301[a] | 6.7 | 9.1 | 4.3 | |
| 302[b] | 9.3 | 12.3 | | 9.2 |

| | All with steroids | | | |
|---|---|---|---|---|
| | **SRL + AZA** | **CsA + AZA** | **SRL + MMF** | **CsA + MMF** |
| 207[c] | 10 | 5 | | |
| 210[d] | | | 5 | 8 |

[a,b,c,d]p = NS.

In a randomized study of the Mayo clinic in which all patients were treated with thymoglobulin, steroids and mycophenolate mofetil and were randomized to receive tacrolimus or sirolimus, the rate of surgical complications was greater with sirolimus, although it decreased once sirolimus target levels decreased, and more obese patients were excluded[231].

Kandaswamy et al. reported the results of a trial of maintenance without steroids in patients randomized to receive two different combinations odf sirolimus plus tacrolimus or cyclosporine plus mycophenolate, finding a higher rate of surgical complications in sirolimus group[232].

The Cleveland group retrospectively reviewed their incidence of healing problems in several immunosuppression cohorts without finding a greater frequency of problems in the sirolimus plus mycophenolate cohort, whereas the risk factor most clearly associated to healing problems was obesity[233].

When sirolimus has been used in lung transplants from the beginning of the transplant, delays have been reported in bronchial anastomosis healing[234]. Problems do not seem to occur when sirolimus is started three months after the transplant[235].

### Recommendations

Several approaches have been successfully used in order to attempt to reduce lymphocele rate. ,Using a delayed start sirolimus and tacrolimus combination, the Nebraska group has found a lymphocele rate of less than 4%[236]. The Texas group reported great improvement in their surgical results in sirolimus regimens dropping from a 38% to a 7% healing delay and/or lymphocele rate when they modified their surgical technique, maintaining drainage for longer periods and using discontinuous suture[237]. The use of delayed sirolimus introduction protocols might contribute towards overcoming these surgical difficulties[171,236,238,239]. Another way of minimizing these complications could be the use of fast steroid elimination protocols[240,241]. In this sense, at the University of Cincinnati, a steroid-free sirolimus protocol using thymoglobulin and mycophenolate has achieved an important reduction in lymphocele rates especially in the more obese population[242].

In terms of major surgeries performed in patients receiving sirolimus it could be wise to temporarily

withdraw the drug and reintroduce it again some days later.

## DIARRHEA

The incidence of diarrhea reported as an adverse event in sirolimus development trials is shown in table XII below. It is observed therein that diarrhea reports were more frequent the greater the exposure to sirolimus and in combinations of the latter with mycophenolate mofetil.

Similarly, in the Cleveland group calcineurin inhibitor-free scheme that used the combination of sirolimus and mycophenolate, a reduction in the MMF dose from 2 grams / day to 1 gram / day was necessary, and consequently a reduction in digestive symptoms such as diarrhea, epigastralgia and nausea was obtained[243]. In calcineurin inhibitor withdrawal and sirolimus introduction trials in which part of the patients was also treated with mycophenolate mofetil, diarrhea occurred in one third of the patients[141].

### Recommendations

This diarrhea occurring as a drug side effect could be handled symptomatically by means of loperamide or octreotide, having reasonably excluded other causes of post-transplant diarrhea such as cytomegalovirus or lymphoma[244].

As in the case of cytopenias, special attention must be paid to possible interactions with mycophenolate mofetil[65-69], it being advisable to either monitor MPA levels or limit the MMF dose to around one gram a day.

**Tabla XII.** % diarrhea with two year follow-up

| | All with cyclosporine and steroids | | | |
|---|---|---|---|---|
| **Studies** | **SRL 2 mg/d** | **SRL 5 mg/d** | **Placebo** | **AZA** |
| 301 (2) | 26 | 38.3[a] | – | 18.1 |
| 302 (2) | 23 | 33[a] | 21 | – |
| | **All with steroids** | | | |
| | **SRL + AZA** | **CsA + AZA** | | |
| 207[b] | 29 | 21 | | |
| | | | **SRL + MMF** | **CsA + MMF** |
| 210[c] | | | 38 | 11 |

[a]p < 0.05 *versus* azathioprine or placebo ; [b]NS; [c]p = 0.008.

## ARTHRALGIAS, OSTEOPENIA AND OSTEONECROSIS

The role of sirolimus on the bone was compared to other immunosuppressants in several preclinical experiments. In contrast with cyclosporine and tacrolimus, which in rats give rise to a loss of high remodeling trabecular bone, sirolimus preserves the volume of spongy bone in treatments of up to a month long[245,246]. Goodman et al. treated several groups of rats with several combinations of immunosuppressants including cyclosporine at high or low doses, or sirolimus at low doses with or without cyclosporine. The association of sirolimus with cyclosporine did not cause the loss of bone mass[247]. Treatment with sirolimus did not produce the increased osteocalcin, a bone turnover marker, observed with cyclosporine and tacrolimus[245,247]. It has been suggested that the sirolimus protecting mechanism on the bone could be based on the increase production of osteoprotegerin, an osteoclast function and development inhibitor, by mature osteoblasts[248]. The effect of sirolimus on PTH levels in murine models seems to be neutral and similar to that of cyclosporine[246,249].

In randomized trials 207 and 210 comparing a sirolimus-based immunosuppression scheme with another one with cyclosporine, bone turnover markers such as serum osteocalcin and urinary telopeptides were measured over 12 months in 115 renal transplant recipients. They received corticoids in both studies. Higher levels of urinary telopeptides and serum osteocalcin were found in patients treated with cyclosporine. These differences were significant at week 24 for telopeptides and at weeks 12, 24 and 52 for osteocalcin[250]. Bone density measurements (DEXA) performed at months 6, 12, 24 and 36 after the transplant in two phase III registry studies (studies 301 and 302 with cyclosporine and corticoids in all the arms) did not find differences in femur neck and lumbar spine bone density between patients treated with sirolimus and those treated with azathioprine or placebo, both in men and women.

The following tables describe the incidence of arthralgias and osteonecrosis, and the analytical bone profile in the main phase II and III studies:

Although the results in the attached tables are not very conclusive, they suggest a discrete increase of

bone necrosis with the higher exposures to sirolimus and a clinically non-significant reduction of calcemia. PTH levels were not systematically determined in these trials.

Campistol et al. reported on 8 renal transplant recipients undergoing treatment with sirolimus with a reflex sympathetic dystrophy-like syndrome (RSDLS) (algodystrophy, bone pain in distal part of members or epiphysis, currently included in the type I complex regional pain syndrome (CRPS)). All the patients had moderate bilateral arthralgias affecting the knees and/or ankles and/or feet and pathological changes compatible with RSDLS in bone scintigraphy. All the laboratory parameters were normal including PTH. The syndrome was resolved in all patients after a mean of 4 months. Treatment consisted in calcitriol (concomitant with calcitonin in one patient) without sirolimus withdrawal[251].

Bhandari et al. communicated two cases of osteonecrosis in renal transplant recipients receiving sirolimus with cyclosporine and steroids. Sirolimus levels were 20.7 ng/mL in one patient and 5.8 ng/mL in the other. They came in with pain in the left leg and hips, and the avascular necrosis diagnosis was confirmed by means of X-rays and magnetic resonance in both hips. In the opinion of the authors, sirolimus would have played a role in the genesis of these osteonecroses due to its early application after transplant (7 and 6 months respectively)[252].

Boardman et al. have recently reported nine cases of patients with sirolimus and bone pains that were described as bone marrow edema, arthritis, osteoporosis or avascular necrosis. The symptoms were handled by means of sirolimus dose reduction or withdrawal (in four of the nine cases)[253].

The observation that sirolimus reduced longitudinal bone growth in rats[245,249] advised monitoring pediatric patients. Preliminary data from the Texas group in eight pediatric patients treated with the sirolimus, cyclosporine and steroid combination do not suggest a special impact on bone growth[254].

## Recommendations

With the current data on the role of sirolimus it does not seem necessary to perform any special changes in usual post-transplant bone care[255,256].

Regarding pain symptoms, their etiology is difficult to characterize. In the past it was proposed that vasoconstriction and hypertension would be the underlying mechanism of several pain symptoms due to cyclosporine and that these would respond to

**Tabla XIII.** % musculoskeletal events reported in sirolimus trials

| | | All with cyclosporine and steroids | | | |
|---|---|---|---|---|---|
| **Trial** | | **SRL 2 mg/d** | **SRL 5 mg/d** | **Placebo** | **AZA** |
| 301 (2) | Arthralgias | 21 | 28[a] | – | 18 |
| | Osteonecrosis | 2,5 | 4,4[b] | – | 0 |
| 302 (2) | Arthralgias | 26 | 26 | 20 | – |
| | Osteonecrosis[c] | 2 | 4 | 0 | – |
| | | **All with steroids** | | | |
| | | **SRL + AZA** | **CsA + AZA** | | |
| 207 | Arthralgias[d] | 19,5 | 0 | | |
| | Osteonecrosis | 2,4 | 0 | | |
| | | | | **SRL + MMF** | **CsA + MMF** |
| 210 | Arthralgias | | | 12,5 | 10,5 |
| | Osteonecrosis | | | 5 | 0 |

[a]p<0.01 versus azathioprine; [b]p<0.05 Fisher exact test; [c]p<0.01

**Tabla XIV.** Analytical results after 1 year

| | | Todos con ciclosporina y esteroides | | | |
|---|---|---|---|---|---|
| **Trial** | **After 1 year** | **SRL 2 mg/d** | **SRL 5 mg/d** | **Placebo** | **AZA** |
| 301 | Calcium (mmol/L) | 2.39 | 2.33[a] | – | 2.42 |
| 302 | Calcium (mmol/L) | 2.47 | 2.39[a.b] | 2.49 | – |
| | | **All with steroids** | | | |
| | | **SRL + AZA** | **CsA + AZA** | | |
| 207 | Calcium (mmol/L)[c] | 2.34 | 2.43 | | |
| | Magnesium (mmol/L) | 0.83 | 0.79 | | |
| | Phosphorus (mmol/L)[d] | 1.01 | 1.11 | | |
| | | | | **SRL + MMF** | **CsA + MMF** |
| 210 | Calcium (mmol/L) | | | 2.42 | 2.42 |
| | Magnesium (mmol/L) | | | 0.82 | 0.74 |
| | Phosphorus (mmol/L)[d] | | | 0.97 | 1.03 |

[a]p < 0.001 compared to azathioprine or placebo; [b]p < 0.001 compared to SRL 2 mg; [c]p < 0.05; d: serum phosphorus levels were significantly lower with sirolimus at several moments during the first year of the transplant.

calcium antagonists. Another painful effect related to calcineurin inhibitors and perhaps also to sirolimus would be the reflex sympathetic dystrophy syndrome (or type I RSDS) which would be accompanied by skin disorders and could respond to immunosuppressant, calcitonin or as has been more recently suggested, diphosphonate, dose reductions. The use of the latter could also contribute towards preserving bone mass, as long as the existence of a low turnover bone disease has been previously ruled out[257].

## INTERSTITIAL PNEUMONITIS, BRONCHIOLITIS OBLITERANS ORGANIZING PNEUMONIA

Morelon et al. communicated for the first time the finding of three cases of interstitial pneumonitis in renal transplant recipients receiving treatment with sirolimus and prednisone, and other different drugs. They showed bilateral infiltrates and the bronchoalveolar lavages did not reveal any microorganisms. The patients improved after withdrawing sirolimus without receiving antibiotics[258]. These same investigators of the Necker hospital of Paris later communicated the existence of five other cases[259]. The bronchoalveolar lavage of the total of eight cases showed a mainly CD4 lymphocyte alveolitis in seven patients and alveolar hemorrhage data in one patient. Transbronchial biopsies were carried out in two patients showing bronchiolitis obliterans data with organizing pneumonia and lymphoid interstitial pneumonia. Sirolimus was suspended in four out of these five new cases and the dose was reduced in one of them. The pneumonitis episode was resolved in all cases in about 3 months. The Necker hospital group has recently updated their case records of sirolimus-related pneumonitis which reached 21 patients. The symptoms appear more frequently in conversions than in de novo uses. Occurrence of lymphocyte alveolitis is a constant, although it might not appear at the beginning of the symptoms. They exhibited sirolimus levels at the time of the symptoms between 12 and 20 ng/mL[260].

The histology is generally compatible with bronchiolitis obliterans with organizing pneumonia in most cases, although cases of alveolar proteinosis or granulomatous disease have been described[260-262].

The association of pneumonitis with high levels of sirolimus has been stressed by several authors[260], but also has been described with normal levels[263].

After the initial report by Morelon et al. the FDA communicated 34 cases of pneumonitis that might have been related to sirolimus up to that time. Of these, there was information ruling out an infectious etiology in only 14 cases, and only in 8 cases was improvement of the symptoms established after withdrawing sirolimus, thus stressing the difficulty of determining the diagnosis of pneumonitis due to sirolimus[262].

Other authors have reported on lung adverse reactions to sirolimus[264-277].

Regarding the usefulness of using steroids in handling these syndromes, the Necker Hospital group used steroids in 1 mg/kg doses in 3 out of 21 patients exhibiting more serious symptoms and an absence of improvement after withdrawing sirolimus[260] (the remaining patients received prednisone doses between 10-30 mg due to its immunosuppressant effects against pneumonia). Other authors have also used steroids to control the course of the disease[261,267,272,274,278,279]. Thus, Henry recommends using steroids if there is no improvement on the grounds of the usefulness thereof in cases of pneumonitis induced by other drugs such as methotrexate[280].

## NEUROTOXICITY

Despite the fact that sirolimus altered astrocyte metabolism in experimental studies[281], Maramatton et al. have reviewed their experience with sirolimus in 202 kidney or liver transplant patients without finding a single case of neurotoxicity after starting sirolimus for a mean treatment duration of 18 months[282].

Therefore, the usefulness of sirolimus has been suggested for salvage treatment in cases of neurotoxicity due to tacrolimus or cyclosporine[90,283-290]. Most of these publications comprise a limited number of cases, but the change is successful in most cases.

Pharmacokinetic interactions have been described with some anticonvulsants such as phenytoin, carbamazepine or phenobarbital which may reduce sirolimus doses[48,49,285,291].

## PREGNANCY

Sirolimus is embryotoxic in rats (at doses 0.2 to 0.5 times the clinical doses used, adjusted for body surface) and this toxicity was manifested as mortality, low weight and ossification delays). In ad-

dition, mTOR knocked-out rats exhibit embryonic development arrested[292]. Teratogenesis was not found. Rats had greater embryo/fetal mortality in combination with cyclosporine. Effects were not observed on the development of rabbits (at doses 0.3 to 0.8 times the clinical doses adjusted for body surface). A case has been communicated in which sirolimus treatment was followed during the first 6 weeks of pregnancy without problems being observed at birth[293]. The FDA includes sirolimus the same as the remaining immunosuppressants in category C for pregnancy risk (adverse effects demonstrated in animals without controlled studies in pregnant women). Given the little information on immunosuppressant use during pregnancy it is important to communicate these cases to the National Transplantation Pregnancy Registry (http://www.centerspan.org/registries/ntpr.htm). Information regarding pregnancies fathered by patients treated with immunosuppressive drugs is even scarcer. A recent report from the NTPR suggests that there is not a higher incidence of congenital defects than in the general population[294].

**Table XV.** Publications of sirolimus use due to calcineurin inhibitor-related neurotoxicity

| Author | N | Source of neurotoxicity | Type of neurotoxicity | % Response to conversion |
|---|---|---|---|---|
| Neff[283] | 11 | Tacrolimus | – Peripheral neuropathy (n = 2)<br>– Confusional syndrome (n = 5)<br>– Aphasia (n = 2)<br>– Cephalea (n = 1)<br>– Tinnitus (n = 1) | Complete 63%<br>Improvement 37% |
| [284, 290] | 4 | Tacrolimus | – Seizures | 100% |
| Hodges[285] | 1 | Cyclosporine | Seizures | Resolved |
| Sundberg[90] | 4 | Cyclosporine or tacrolimus | Not specified | 75% |
| Toth[286] | 1 | Tacrolimus | Migraine | Resolved |
| [287] | 10 | Tacrolimus | – Tremors<br>– Cephaleas | 100% |
| Forgacs[288] | 7 | Tacrolimus | – Peripheral neuropathy (n = 4)<br>– Seizures (n = 2)<br>– Encephalopathy (n = 1)<br>– Central pontine myelinolysis (n = 1) | 100 |
| [289] | 3 | Tacrolimus | | 100 |

The low frequency of reporting of these events advises to extreme precautions when taking this information into account.

The current summary of product characteristics meanwhile recommends avoiding pregnancy during treatment with sirolimus and 12 weeks after suspension thereof[49]. In a short study no clinically significant pharmacokinetic interaction between sirolimus oral solution and norgestrel – ethinyl estradiol was found.

Recently, a report from a consensus conference on reproductive issues and transplantation has been published. Several recommendations are extended, and a great number of current uncertainties are underlined[295].

## NEOPLASMS

At least from a preclinical point of view, sirolimus, unlike calcineurin inhibitors, does not promote tumor development. Geissler et al. have recently reviewed antitumoral mechanisms of sirolimus that include cell cycle arrest, antiangiogenic effect, promotion of tumoral cell apoptosis, and inhibition of phophatidil-inositol kinase pathway and several transcription factors[296].

From a clinical perspective, Mathew et al. reported the 2-year incidence of tumors in five randomized trials with sirolimus «de novo», which is lower in patients treated with sirolimus-based schemes or in those with cyclosporine withdrawal than in control groups[297]. This benefit is observed in skin as well in non-skin tumors[298]. Likewise, the Texas´s team has reported a low incidence of postransplantion lymphomas in the cohort of patients receiving sirolimus[299]. A retrospective registry from the UNOS database suggests that the use of mTOR inhibitors alone or in combination with a calcineurin inhibitor as discharge maintenance immunosuppression was associated with a significantly reduced incidence of post-transplant de novo malignancy within two years follow-up[300].

Switching to sirolimus has been successfully used as a non-specific treatment for postransplant tumors in several non-controlled experiences such as in Kaposi´s sarcoma[301-303]), relapsing skin squamous cell carcinoma[304], hepatocarcinoma[305,306], or lymphoma[307-310].

These data have advocated the use of sirolimus-based schemes among an avoidance of lymphocyte depleting antibodies and steroids minimization as an approach to decrease tumoral relapse incidence in those patients with a malignancy history pretransplantation[311].

**Table XVI.** Recomendations

*Management of sirolimus-induced hiperlipidemia*
- Adjust sirolimus trough levels
- Fulfill suggestions from the Panel III of NCEP or K/ DOQI guidelines 46, 47
- Changes in life style
- Target LDL < 100 mg/dL and cholesterol < 200 mg/dL
- Use of ezetimibe in cases of statin resistance

*Management of sirolimus-induced anemia*
- Adjust sirolimus trough levels
- Measure MPA levels in conversions or limit dose of MMF to a maximum of 1 gr/day.
- Temporarily use of erythropoietin
- Measure ferritin status to rule out unexpected ferropenic states

*Management of sirolimus-induced leucopenia or thrombocytopenia*
- Adjust sirolimus trough levels o watchful waiting to self-correction
- Measure MPA levels in conversions or limit dose of MMF to a maximum of 1 gr/day.
- Temporarily use of G-CSF (leucopenia).

*Management of thrombotic microangiopathy*
- If the patient is receiving calcineurin inhibitors consider withdrawing them if possible as first choice. If the patient is receiving a CNI-free regimen, consider withdrawing sirolimus if feasible. .
- Treatment of concomitant factors such as CMV, or acute rejection

*Management of infections while treated with sirolimus*
- Implement prophylaxis for P. Jirovecii during 6 months with cotrimoxazole during 6 months in the «de novo» patient.
- No other changes in the usual antibacterial or antiviral infective policy

*Management of sirolimus-induced edema*
- Adjust sirolimus trough levels
- Consider changes in anti-hypertensive drugs such as calcium blockers withdrawal
- Test low doses of diuretics although response is probably not optimal.

*Management of renal function with sirolimus*
- The concomitant use of sirolimus plus CNI should be limited in time (see chapter of «de novo» use and «conversion» in this monograph)
- Delayed introduction of sirolimus in patients with acute tubular necrosis
- Do not convert patients with important baseline proteinuria (see chapter on conversion)

*Management of sirolimus-induced oral ulcers*
- Mouthwash with sucralfate, lidocaine o topical steroids
- Adjust sirolimus trough levels or withdraw in cases of severe intolerance
- Unlikely utility of changes between oral solution and tablets.

*Management of surgical issues with sirolimus*
- Delayed introduction of sirolimus (7-14 days) in patients specially at risk (obesity, diabetes)
- Extreme surgical techniques: drainages, interrupted wound closure
- Explore steroids – free protocols in future trials
- In the case of new major surgery required, adjust sirolimus levels and consider temporarily withdrawal («sirolimus holidays») if feasible.

*Management of sirolimus-related diarrheas*
- Measure MPA levels in conversions or limit dose of MMF to a maximum of 1 gr/day.
- Symptomatic treatment with loperamide
- Decrease sirolimus doses or temporal withdrawal. Possible utility of dividing total amount of drug in two doses each 12 hours

*Management of osteomuscular problems with sirolimus*
- No special changes in usual bone protecting policies
- Management of arthralgias with decreasing of doses or calcitonin or diphosphonates or symptomatic treatment .

*What to do in pregnancy*
- As in almost all immunosuppressants there is not enough experience to set recommendations. Consider temporarily withdrawal if feasible.

*Management of sirolimus-associated lung toxicity*
- Exclude infectious causes: cultures, bronchoalveolar lavages, thorax CT.
- Adjust sirolimus trough levels and consider withdrawing taking into account the severity of the picture.
- Steroids use in more severe cases could be a choice although the evidence are very weak

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