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Benefit-Risk Assessment of Ciclosporin Withdrawal in Renal Transplant Recipients

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Abstract

Ciclosporin is associated with significant toxicity, including nephrotoxicity, and with an increased risk of cardiovascular events. Many attempts have been made to wean patients from ciclosporin. Before the availability of new immunosuppressive drugs, the acute rejection rate observed after ciclosporin withdrawal did not permit the widespread use of withdrawal regimens even though meta-analysis did not show that they adversely affected patient or graft survival. Nevertheless, maintenance therapy with azathioprine and corticosteroids has not become routine practice. The introduction of mycophenolate mofetil and subsequently sirolimus has increased the number of clinical studies of the effects of ciclosporin withdrawal.

In stable patients, this withdrawal is associated with a small but significant increase in the incidence of acute rejection episodes. Declining renal function and

other forms of ciclosporin-related toxicity have improved. However, this improvement was also observed when ciclosporin was only reduced (and not withdrawn), which did not increase the risk of acute rejection. More precise definition of the patients who could benefit from ciclosporin-withdrawal may help to optimise the immunosuppressive regimen in this setting.

In patients with chronic allograft deterioration, ciclosporin withdrawal together with mycophenolate mofetil introduction has been shown to improve renal function significantly in many small studies, and a large prospective randomised study. For the time being, ciclosporin withdrawal is a good therapeutic option for patients with declining renal function and signs of chronic ciclosporin nephrotoxicity on renal biopsy.

Finally, recent preliminary studies have reported the results of complete avoidance of calcineurin inhibitors after renal transplantation. These results are promising as regards the incidence of acute rejection, renal function and safety, but need confirmation in larger trials with a longer follow-up.

Nevertheless, it has become clear that the concept of an immunosuppressive regimen with little or no nephrotoxicity after renal transplantation is more and more important and plays a crucial part in tailoring immunosuppression to the needs of specific patient populations.

Since the early 1980s, standard care for immunosuppression in transplant recipients has involved the use of calcineurin inhibitors such as ciclosporin and tacrolimus. Ciclosporin inhibits T-cell activation pathways which require a rise in the intracellular free calcium concentration, thus reducing the production of interleukin (IL)-2.[1] Ciclosporin binds specifically and with high affinity to a family of receptors called cyclophilins.[2,3] This drug-receptor complex inhibits the activation of calcineurin phosphatase which dephosphorylates and activates the nuclear factor of activation of T cells (NF-AT). NF-AT increases transcription of IL-2. Inhibition of IL-2 transcription by ciclosporin therefore stops the proliferation and activation of helper and cytotoxic T cells.[4] The results of several studies suggest that the use of ciclosporin after renal transplantation reduces the number of acute rejection episodes and enhances short-term allograft survival.[5] However, most of the benefit appears to result from a decrease in the number of acute rejections during the first months after transplantation. After the first post-transplant year, the rate of graft rejection appears to be no different, whether or not patients are treated with ciclosporin. Ciclosporin is indeed associated with significant nephrotoxic adverse effects in the short- and long-term.[6-10] The balance between preventing immunological allograft failures and managing nephrotoxicity is still an unsolved issue. In order to include the non-immunological components of late graft loss or renal dysfunction after kidney transplantation, especially drug-induced nephrotoxicity, the term ‘chronic rejection’ has been replaced by ‘chronic allograft nephropathy’. In an attempt to improve the long-term survival of grafts and patients, and to avoid ciclosporin-related adverse effects, conversion from ciclosporin to other non-nephrotoxic immunosuppressive drugs has been extensively reported in the literature since 1988. The recent introduction of new immunosuppressive drugs such as mycophenolate mofetil or sirolimus has strengthened the case for minimising the use of ciclosporin. The main strategies for reducing potential ciclosporin-induced nephrotoxicity consist of sparing ciclosporin in induction regimens from the day of transplantation or eliminating it

altogether, and ciclosporin reduction or withdrawal from maintenance regimens. Nevertheless, even though the other approaches are of interest, the present benefit-risk assessment will focus on the results of complete ciclosporin withdrawal from maintenance treatment after renal transplantation.

1. Benefit and Risk Definition

1.1 Benefit: Reduction of Ciclosporin-Induced Toxicity

1.1.1 Ciclosporin-Induced Nephrotoxicity

Nephrotoxicity is the major adverse effect of ciclosporin. In both experimental models and human clinical studies, it has been well established that ciclosporin produces dose-dependent, acute and reversible vasoconstriction of renal arterioles. Ciclosporin-induced acute renal failure may occur as early as a few days or months after the initiation of therapy.[11] The clinical manifestations of renal arteriolar vasoconstriction or acute renal dysfunction include reduction of the glomerular filtration rate (GFR), hypertension, hyperkaliemia, tubular acidosis, increased reabsorption of sodium and oliguria.[12] The adverse effects on renal haemodynamics are thought to be directly related to blood ciclosporin concentrations. The precise mechanism and the mediators involved in these adverse effects and implicated in the alteration of renal haemodynamics have yet to be identified. Many mediators such as endothelin, thromboxane A_2, renal prostaglandins and nitric oxide are probably implicated.[13-15] Therapeutic drug monitoring usually helps to reduce the risk of toxicity.

Chronic progressive nephrotoxicity is the major toxic effect in the long term. The transition from acute haemodynamic changes to chronic injury has not been clearly established. Studies based on biopsies of experimental models, patients with autoimmune diseases and extrarenal solid organ transplants have demonstrated the specific pathological and morphological changes occurring in ciclosporin-induced chronic progressive nephropathy.[16-19] Histologically, the latter is characterised by arterial wall destruction, myointimal necrosis and gradual narrowing of the arterial lumen. It is also associated with tubulointerstitial fibrosis with a striped pattern. Unlike acute renal impairment, chronic progressive nephropathy does not seem to be dose dependent. In most cases, it is associated with mild-to-moderate renal dysfunction. Usually, renal function, as estimated by serum creatinine level, has been used as a surrogate marker for the presence of chronic ciclosporin-induced nephropathy. Its pathogenesis is unclear. Low-grade chronic ischaemia due to continuous renal vasconstriction may be an important cause. However, in an experimental model in salt-depleted rats, no clear relationship between glomerular haemodynamics and the development of histological lesions was found.[20] In this model, nitric oxide inhibition by ciclosporin was shown to be important. Other experiments led to the conclusions that the renin angiotensin system is activated intrarenally by ciclosporin, which leads to the deposition of excess matrix protein by stimulating its production and/or diminishing its breakdown.[21] Transforming growth factor (TGF)-β1, has been suggested to play role in causing chronic progressive ciclosporin-induced nephropathy, by acting as a profibrotic cytokines.

The incidence of this nephropathy after renal transplantation is difficult to evaluate. Several processes can lead to tubulointerstitial fibrosis and structural and functional deterioration of the kidney. These include chronic rejection, hypertension, hyperglycaemia, hyperlipidaemia, recurrence of underlying disease and ageing. The major drawback of many studies is the absence of renal histology. In the early 1980s, initial studies have compared the renal function in patients treated with azathioprine or ciclosporin for periods of 1–4 years.[22] The average serum creatinine was 2.1 mg/dL in the ciclosporin group and 1.5 mg/dL in the azathioprine group. Renal allograft biopsies revealed interstitial fibrosis and tubular atrophy in the ciclosporin group. How-

ever, in a large cohort of 1663 renal transplant recipients followed up for 4 years, there was no major evidence of chronic ciclosporin nephropathy.[23] The role of this nephropathy in the course of chronic allograft failure leading to graft loss is still unknown. Despite more than 15 years of ciclosporin-based immunosuppression in renal transplant recipients, there are no prospective studies available that document either the long-term safety of ciclosporin or the effects of ciclosporin-induced structural and functional renal changes on the longevity of renal transplants.

Another approach evaluating the incidence of chronic allograft ciclosporin-induced nephropathy is to examine the occurrence of this complication among non-renal transplant recipients. In one study of heart transplantation, no ciclosporin-treated patient exhibited normal renal function after 36 months. After 7 years, the average serum creatinine level was still increased, at 3.3 mg/dL compared with 1.2 mg/dL at the time of initial hospital discharge.[24] In addition, a 10% prevalence of calcineurin inhibitor-induced end-stage renal failure was reported in heart transplant recipients after 5–10 years of immunosuppressive therapy. A recent randomised trial, in which the effects of two calcineurin inhibitors were compared following liver transplantation, has reported the incidence of renal dysfunction in 305 ciclosporin-treated patients. Renal dysfunction, defined as a serum creatinine level >140 mmol/L, occurred in 50% of patients, and 15% needed renal support.[25] When renal biopsies were performed in liver transplant recipients because of chronic renal impairment, chronic ciclosporin nephrotoxicity was present in about one-third these biopsies. However, many of these non-renal patients had severely impaired renal function preoperatively, and were given high doses of calcineurin inhibitors.

In a meta-analysis and morphological review of ciclosporin-induced nephrotoxicity in autoimmune diseases, Vercauteren et al. reported a difference of 20.9% between the risk of developing nephrotoxicity with ciclosporin therapy and with an alternative therapy.[26] In studies of pre- and post-treatment biopsies, the authors reported either an increase in the number of renal morphological lesions or their development.[27]

The studies in which the renal function of transplant recipients is compared before and after ciclosporin withdrawal are the most informative regarding the relationship between ciclosporin administration and impaired renal function, and will be discussed further in this review.

1.1.2 Other Ciclosporin-Related Toxicities

Ciclosporin has been associated with toxicities other than renal.[28] The most important is the worsening of many cardiovascular risk factors.[29] Thus, ciclosporin is associated with hypertension: its incidence among renal transplant recipients was 50% before the ciclosporin era and is now reported to be >80%. Hyperlipidaemia is another common cardiovascular risk factor in patients treated with ciclosporin. Ciclosporin also induces hypertrichosis and gum hyperplasia, especially in young children.[30] These complications have prompted some authors to switch patients from ciclosporin to another calcineurin inhibitor, namely tacrolimus. Since cardiovascular disease is the most frequent cause of death, and is also a cause of allograft failure, these issues are becoming increasingly important, as the improvement of immunological complications is observed after renal transplantation.

1.2 Risk Evaluation

The major risks after ciclosporin withdrawal are the development of acute or chronic rejection. The diagnosis of acute rejection is often easy, as this rejection is characterised by a rise in serum creatinine in a previously stable patient. In that case, a renal biopsy is indicated, to confirm both the diagnosis and reveal the extent of the lesion.[31] However, the long-term risk may be the appearance of chronic allograft nephropathy. Only long-term follow-up of patient cohorts will address this issue. Some surrogate markers such as routine renal biopsies may also be used.[32] In one prospective pilot

study, ciclosporin withdrawal was associated with chronic histological deterioration in 50% of patients despite a low incidence of acute rejection.[33] Furthermore, in this study, the biopsies of patients whose renal function did not improve after ciclosporin withdrawal exhibited chronic lesions.

2. Other Immunosuppressive Treatment

To permit ciclosporin withdrawal, other active drugs have to be used for effective prevention of acute rejection.

Corticosteroids remain an important part of immunosuppressive treatment. Their mechanism of action and the complications they may cause will not be reviewed here. However, it should be noted that corticosteroid withdrawal too has been tested for many years, and can be an alternative to ciclosporin withdrawal, depending on the adverse effects experienced by individual patients.[34,35] Even though some authors have studied monotherapy-based immunosuppression,[36] combined corticosteroid and ciclosporin withdrawal has never been tested on a large scale.

2.1 Tacrolimus

Tacrolimus also acts through calcineurin inhibition and is therefore used as an alternative to ciclosporin. For some patients who experience specific ciclosporin adverse effects, such as cosmetic problems or thrombotic microangiopathy, conversion from ciclosporin to tacrolimus may be beneficial.[37,38] However, since tacrolimus has the same mechanism of action as ciclosporin and is associated with significant nephrotoxicity, we will not discuss this conversion in the present review.

2.2 Azathioprine

Azathioprine is a purine synthesis inhibitor, which has been extensively used for more than 30 years to prevent acute rejection episodes after renal transplantation. It acts by inhibiting both *de novo* and the salvage pathway of purine synthesis, possibly by incorporating pseudo-nucleotides into DNA.[39] Today, azathioprine is not often used for *de novo* renal transplant recipients because of the lack of controlled information regarding its efficacy and the introduction of newer immunosuppressive drugs which were have proved to be more effective.[40]

2.3 Mycophenolate Mofetil

Mycophenolate mofetil is a prodrug of mycophenolic acid, a potent, non-competitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH is the key rate-limiting enzyme of the *de novo* pathway for the synthesis of purine, specifically guanosine. Mycophenolate mofetil therefore inhibits the cell cycle especially lymphocyte proliferation, and T-lymphocytes are induced more dependent on this pathway than other cell types.[41] Mycophenolate mofetil has been shown to be more effective than azathioprine in preventing acute graft rejection, in both kidney transplant recipients and other organ transplantation.[42] Some authors have also shown that mycophenolate mofetil may reduce late renal allograft loss.[43]

2.4 Sirolimus

Sirolimus (rapamycin) is a macrocyclic lactone, a fermentation product of *Streptomyces hygroscopicus*. Sirolimus has a mechanism of action distinct from that of ciclosporin. Sirolimus reduces T-cell activation at a later stage in the cell cycle than ciclosporin. It cross-links the immunophilin FK binding protein (FKBP) 12, a peptide-prolyl isomerase that acts as a folding catalyst, with the multifunctional serine-threonine kinase, the mammalian target of rapamycin (mTOR). Blockade of mTOR dampens lymphocyte responses to costimulatory signal 2 during the G_0 to G_1 transition, and to cytokine signal 3 during the G_1 build-up, and suppresses IL-2- or IL-4-driven T-cell proliferation.[44] Large phase III trials have shown that sirolimus is a potent immunosuppressive drug that prevents acute rejection episodes. The combination of sirolimus

and ciclosporin has displayed synergistic interaction to significantly reduce the incidence of acute graft rejection in renal allograft recipients.[45,46] However, patients given this combination have also experienced a range of non-immune toxicities, including potentiation of certain ciclosporin-related adverse reactions such as renal dysfunction, even though in animals, sirolimus was found to be devoid of nephrotoxicity in animals when given alone.

3. Ciclosporin Withdrawal in the Azathioprine Era

The most comprehensive reviews of long-term evaluation of ciclosporin withdrawal during the azathioprine era are the two meta-analyses by Kasiske et al.[47,48] The first was published in 1993, and dealt with elective ciclosporin withdrawal after renal transplantation, for which the authors scanned the literature using the National Library of Medicine Medline database, and cited 64 investigations.[47] They adopted three criteria for the inclusion of a study in their analysis: first, it must concern a group of renal transplant recipients for whom ciclosporin had been electively discontinued. Secondly, it must include a suitable control group of patients who had never been given ciclosporin; and lastly, it must give precise data on actual or actuarial patient and/or graft survival. Studies were excluded if the reason for withdrawal was prolonged delayed graft function and/or severe acute rejection. Although 33 of the publications reported controlled studies, 22 were uncontrolled. The assessment criteria reduced the 64 publications to 17 studies that were suitable for meta-analysis.

The authors first analysed the effects of withdrawal versus continued ciclosporin therapy. The mean duration of follow-up after transplantation was 26.6 ± 7.5 months (range: 12–36). The minimum follow-up time was 14.7 ± 6.5 months (range: 4–24) and the maximum, 31.0 ± 10.5 (range: 24–49). There was a higher rate of acute rejection in patients after withdrawal than in control patients who continued on ciclosporin ($p < 0.001$). However, in the largest study included in the analysis, in which 113 patients underwent withdrawal, and 138 continued on ciclosporin, there was no increase in the acute rejection rate.[49] In none of the individual studies analysed was there a significantly higher rate of graft loss among patients for whom ciclosporin was withdrawn than among control patients who continued ciclosporin treatment. Kasiske et al. reported that in the larger controlled trials, there appeared to be a trend towards a reduction of the difference between the graft loss of patients whose ciclosporin was withdrawn and control patients. No differences were observed if only first transplants were included, if living related transplant recipients were included, if ciclosporin was discontinued early rather than late, if ciclosporin was discontinued abruptly or if the corticosteroid dose was increased before or during the tapering of ciclosporin. Neither was there any difference between the mortality of the ciclosporin withdrawal and control groups. The serum creatinine level tended to be higher in patients who continued on ciclosporin than those for whom it had been withdrawn (163 ± 26 μmol/L vs 144 ± 25 μmol/L; $p = 0.17$).

The second part of this meta-analysis by Kasiske et al. concerned studies in which the effects of ciclosporin withdrawal were compared with those of 'conventional' immunosuppression without ciclosporin. In almost all the studies concerned, ciclosporin was withdrawn during the first 6 months after transplantation and the withdrawal was either sudden or tapered over 2–4 weeks. The mean duration of follow-up was 28.8 ± 11.6 months (range: 11–38). The rate of rejection was significantly higher in the patients whose ciclosporin was withdrawn than in the controls ($p = 0.04$). The rate of graft loss was not significantly different in the two groups, neither were the differences between their respective mortality rates. Lastly, mean serum creatinine levels tended to be higher in the control patients who had never been given ciclosporin than in the patients whose ciclosporin had been withdrawn (151 ± 32 μmol/L vs 133 ± 16 μmol/L; $p = 0.20$).

Since this meta-analysis had been criticised for the relatively short length of follow-up, the authors published a second meta-analysis of the results of ciclosporin withdrawal in trials involving renal transplant recipients.[48] Their results confirmed those of the previous analysis. For acute rejection, the pooled mean difference between ciclosporin withdrawal and conventional immunosuppression was 0.11 ($p < 0.001$), indicating that the proportion of patients with acute rejection after ciclosporin withdrawal was 11% higher. Except for the characteristics of the study, no factor was found to be predictive of poor outcome. The relative risk of graft failure was not different in the ciclosporin withdrawal and control groups. Interestingly, in the studies with a short follow-up, ciclosporin withdrawal was more likely to have an adverse effect on graft survival than in studies with a longer follow-up.

The total dollar cost of immunosuppression is substantially reduced by the withdrawal of ciclosporin treatment.[50-52] In the ciclosporin withdrawal study by Hollander et al.,[53] the frequency of cardiovascular death with a functioning graft was found to be 8% higher in the group remaining on ciclosporin. This difference only became apparent 5 years after ciclosporin discontinuation. In a monocentric retrospective study from Minneapolis, there was no difference between the graft or patient survival with or without ciclosporin-elective withdrawal.[54] Even after adjusting for multiple-risk factors, the decision to withdraw or continue ciclosporin had no statistically significant effect on graft failure, death-censored graft failure or death with a functioning graft.

Studies of living-related donor graft recipients have shown that withdrawal is safer and more economical than long-term administration of low-dose ciclosporin.[55,56] However, in the first of these studies, a high rate of rejection was observed, especially in the rapid withdrawal group.[55]

4. Modern Era: Ciclosporin Withdrawal in Stable Patients

4.1 Ciclosporin Withdrawal with Mycophenolate Mofetil in Stable Patients

Since 1995, new immunosuppressive drugs have been approved for the prevention of acute rejection after renal transplantation. Beside new induction agents such as anti-IL2-receptor monoclonal antibodies, mycophenolate mofetil and sirolimus have been shown to be potent immunosuppressive drugs. Mycophenolate mofetil and sirolimus have both been shown to reduce the acute rejection rate compared with a placebo or azathioprine during the initial period after transplantation, when used together with ciclosporin and corticosteroids.[42]

Because of the absence of nephrotoxicity, mycophenolate mofetil has been investigated in an attempt to improve the outcome of ciclosporin withdrawal in renal transplantation. A preliminary report has been published in 2000.[57] In 17 patients whose renal function had been stable for more than 6 months, a conversion to mycophenolate mofetil was combined with stepwise withdrawal of ciclosporin. The mean time after transplantation was 26 months (range: 7–117). Mycophenolate mofetil introduction was associated with severe diarrhoea in one patient, and an acute rejection episode occurred in one patient during the low-dose ciclosporin step. No other rejection was observed after complete ciclosporin withdrawal. Lowering ciclosporin daily dose (i.e. mean ciclosporin trough level decreased from 130 to 45 μg/L) was associated with a 10% improvement in serum creatinine and a decrease in total cholesterol. The GFR increased in 15 patients, from 46.6 ± 8.8 to 58 ± 10.5 mL/min ($p < 0.01$). Renal blood flow also increased significantly associated with a decrease in renal vascular resistance whereas the filtration fraction remained unchanged. During the 1-year follow-up, two other patients experienced a 20–40% rise in serum creatinine, with borderline rejection on renal biopsy in one of the two patients.

In a prospective trial, another group in The Netherlands explored the viability of ciclosporin withdrawal with conversion either to azathioprine or mycophenolate mofetil.[58] Sixty-four patients treated with ciclosporin and prednisone were included in this prospective trial. Only patients with stable graft function 1 year after transplantation, and without acute rejection within the previous 6 months, were randomised for conversion to mycophenolate mofetil (n = 34) or to azathioprine and prednisone (n = 30). The starting daily doses were 2g for mycophenolate mofetil and 2 mg/kg for azathioprine. The ciclosporin dose was gradually tapered and stopped after 4 weeks, without altering the prednisone 10mg dose. After conversion, serum creatinine declined in the mycophenolate mofetil group from 132 to 109 μmol/L (p = 0.02) and in the azathioprine group from 123 to 112 μmol/L. Complete withdrawal proved to be possible in 67% of patients in the azathioprine group and 71% of the mycophenolate mofetil group. After conversion, more acute rejection episodes occurred in the azathioprine group than the mycophenolate mofetil group (37% vs 12% respectively, p = 0.04). A dose reduction of mycophenolate mofetil was necessary in 72% of patients.

Since ciclosporin withdrawal was safer when patients were treated with mycophenolate mofetil than with azathioprine, the same authors also reported the respective effects of ciclosporin or prednisone withdrawal.[59] In this prospective randomised multicentre study involving 212 patients, stable patients were randomised to stop either ciclosporin (n = 63), or prednisone (n = 76), or continue triple drug therapy with mycophenolate mofetil (n = 73) 6 months after renal transplantation. Stable patients were defined as patients with no clinical or biopsy-proven acute or chronic rejection, patients with no proteinuria or unstable renal function. Of interest, these 212 patients were selected among 313 patients, so that only 68% of the preselected patients could effectively enter this prospective trial. The follow-up period was 24 months after transplantation (18 months after inclusion). Ciclosporin was stopped over a period of 2 weeks, with an initial 50% decrease in the daily dose. However, in nine patients, this decrease was slower and complete withdrawal took 9 weeks. In the prednisone-discontinuation group, dosages were tapered to 0mg in 10 weeks.

The incidence of biopsy-proven acute rejection episodes was significantly higher in the ciclosporin-withdrawal group than in the corticosteroid-withdrawal or triple-therapy groups (22% vs 3.9% and 1.4%, respectively). Of the nine patients whose ciclosporin dosage was tapered over 9 weeks, only one patient developed biopsy-proven acute rejection. A second acute rejection occurred in five patients in the ciclosporin withdrawal group versus one patient in the prednisone group. Assessment of the histological evidence of chronic rejection or ciclosporin nephrotoxicity on renal biopsy showed that the incidence of biopsy-proven chronic rejection was 9 of 63 (14.3%) in the ciclosporin withdrawal group versus 4 of 76 (5.3%, p = NS) in the prednisone withdrawal group and 1 of 73 (1.4%, p = 0.006 vs the ciclosporin withdrawal group) in the control group.

Histological changes compatible with ciclosporin nephrotoxicity were present in one patient in the ciclosporin withdrawal group, in three patients in the prednisone withdrawal group, and in four patients in the control group. Noteworthy, no routine biopsies were performed in this study. There was no statistically significant difference between the serum creatinine level or creatinine clearance in the three groups at any time after follow-up. However, assessments of patients who had really been weaned of calcineurin inhibitors (76% of the population), showed significant changes in serum creatinine 12 and 24 months after transplantation. No significant changes in proteinuria were observed in any groups. Mean arterial pressure was not different in the triple therapy and ciclosporin withdrawal groups, and only the prednisone withdrawal group had a statistically significant reduction in mean arterial pressure. Withdrawal of either ciclosporin or prednisone was followed by a rapid fall in total cholesterol levels.

In another prospective controlled study, the effects of ciclosporin and mycophenolate mofetil withdrawal were compared in 84 stable renal transplant recipients who were randomised at 3 months for withdrawal of either ciclosporin (n = 44) over 6 weeks (with two dose reductions of 33%) or of mycophenolate mofetil (n = 40) over a period of 6 weeks (with 500mg dose reduction every 2 weeks).[60] To be eligible, patients had to be in a stable condition, defined by a serum creatinine level <200 mmol/L at 3 months, and to have experienced not more than one acute rejection episode post-transplant, or one episode during the month before study entry. In the ciclosporin-withdrawal group, calcineurin inhibitors had to be reintroduced in three patients (6.8%). Acute rejection episodes occurred more frequently after withdrawal of ciclosporin than mycophenolate mofetil (11.3% vs 5%, p = NS).

At 12 months, patient and graft survival were 100% and 97%, respectively. At 1 year, renal function, assessed by serum creatinine levels and calculated creatinine clearance, was significantly better in the ciclosporin- than the mycophenolate mofetil-withdrawal group. No differences were seen in proteinuria levels. Regarding blood pressure, discontinuation of ciclosporin was associated with a significant beneficial effect. Significantly more patients on ciclosporin than mycophenolate mofetil were using HMG-CoA reductase inhibitors 1 year after transplantation. The difference between serum cholesterol levels was not significant. After ciclosporin withdrawal, hypertriglyceridaemia improved and after mycophenolate mofetil withdrawal, serum triglycerides were almost within the normal range.

Lastly, the effects of ciclosporin withdrawal from an immunosuppressive regimen containing mycophenolate mofetil were assessed in a large open, randomised, controlled study in stable patients with a longer delay after renal transplantation.[61] To be included, patients had to have been undergone transplantation 12–30 months previously, and to have been on triple therapy with ciclosporin, mycophenolate mofetil and corticosteroids for at least 3 months, to have had no more than one rejection episode after transplantation and no rejection episodes during the 3 months before enrolment and to have had stable serum creatinine levels <300 μmol/L for at least 3 months before study entry. In the withdrawal group, ciclosporin was slowly tapered, by thirds of the baseline dosage and withdrawn over a period of 3 months. A total of 170 patients were randomised to one of the groups. Renal function, assessed by the serum creatinine level and creatinine clearance improved, but not significantly, in the ciclosporin withdrawal group. This improvement mostly occurred shortly after ciclosporin tapering began, and remained stable after the last phase of tapering. This improvement probably reflects a reversal of the renal vasoconstriction thought to be caused by ciclosporin.[62,63] Interestingly, creatinine clearance improved by >10% in 46% of the patients in the ciclosporin-withdrawal group.

When only patients of per-protocol population (with effective ciclosporin withdrawal) were considered, there was a statistically significant improvement in creatinine clearance (7.5 mL/min, p = 0.02) in favour of the ciclosporin-withdrawal versus the control group. There was also a significant difference in total and low-density lipoprotein cholesterol between the groups in favour of the ciclosporin withdrawal group, 9 months after randomisation. Blood pressure improved in both groups during the study period. Follow-up of the key parameters will be continued for 5 years after trial entry. In this trial, acute rejection episodes were more frequent after ciclosporin withdrawal than ciclosporin continuation. Nine patients in the withdrawal group experienced 11 rejection episodes (two had two acute rejection episodes) versus two in the ciclosporin-continuation group (p = 0.03). Two rejection episodes occurred late in the ciclosporin-tapering period, when ciclosporin dosage was one-third of baseline, and the other episodes occurred within the first 70 days after complete ciclosporin discontinuation. Three patients required antibody treatment. An attempt was made to analyse the characteristics of

the patients with ciclosporin withdrawal who experienced either acute rejection or no rejection. These characteristics included mean corticosteroid and mycophenolate mofetil dosages, type of graft, cold ischaemic time, donor age, HLA mismatches and panel-reactive antibodies. None of these characteristics was different in the two groups. However, the authors suggested the possible role of slightly lower corticosteroid and mycophenolate mofetil dosage in the patients with rejection. It is noteworthy that the mean daily doses of corticosteroids throughout the study in the ciclosporin-continuation and ciclosporin-withdrawal groups were 7.5 and 13.0mg, respectively ($p = 0.0001$).

Long-term follow-up is mandatory to appreciate the benefit-risk ratio of ciclosporin withdrawal. In a pilot study among stable patients, we reported that 50% of patients experienced significant worsening of chronic lesions on their routine allograft biopsies.[33]

4.2 Ciclosporin Withdrawal with Sirolimus in Stable Patients

Sirolimus is devoid of nephrotoxicity in animal models when given alone. However, sirolimus increased the nephrotoxicity of ciclosporin in a rat model.[64] Furthermore, in double-blind phase III trials, comparison of the effects of sirolimus and azathioprine or placebo in a regimen comprising full-dose ciclosporin and corticosteroids, showed that renal function worsened in patients in the sirolimus-ciclosporin arms.[45,46] An approach, that has been explored is the use of sirolimus with a concentration-controlled design to allow early ciclosporin elimination early from a sirolimus-ciclosporin-corticosteroid regimen.[65,66]

The first study came from a group of 57 renal transplantation centres in Europe, Canada and Australia.[65] The patients enrolled in this prospective randomised, open-label trial received sirolimus, ciclosporin and corticosteroids up to the time of randomisation at month 3 after transplantation. To be randomised, patients had to have experienced no grade 3 acute or vascular rejection during the 4 weeks before randomisation, and to have a serum creatinine level that never exceeded 400 μmol/L. In all, 525 patients were enrolled but only 430 (82%) met the criteria for randomisation at 3 months. Patients were randomly assigned, either to remain on triple-drug therapy including sirolimus (trough levels >5 μg/L) and ciclosporin (trough levels 75–200 μg/L), or to ciclosporin withdrawal. Those in the withdrawal group had their daily sirolimus dose adjusted to maintain trough concentrations of 20–30 μg/L, and the ciclosporin dose was gradually decreased and then eliminated over 4–6 weeks. Ciclosporin withdrawal was successful in 93% of the patients assigned. The median ciclosporin elimination period was 41 days.

One year after randomisation, renal function, measured by either serum creatinine level or calculated GFR was significantly better when ciclosporin was withdrawn. This difference was observed as early as 1 month after randomisation. GFR progressively improved during month 12 in the ciclosporin-withdrawal group. Although some patients who remained on ciclosporin also improved, a larger proportion improved in the ciclosporin-withdrawal group (72.2% vs 40.4, respectively, $p < 0.001$). When patients were grouped according to baseline serum creatinine level (i.e. last value measured before randomisation), all groups potentially benefited from ciclosporin elimination at 3 months, irrespective of their serum creatinine level at this time.[67] On the other hand, >25% of the patients in the ciclosporin withdrawal group experienced no improvement in renal function. After 2 years of follow-up, serum creatinine level was still significantly lower in the ciclosporin-withdrawal group.[68]

Some of the adverse events were less frequent in the ciclosporin-withdrawal group. Thus, hypertension was reported in 7.0% of the patients in the withdrawal group versus 16.3% in the triple-therapy group. In addition, both diastolic and systolic blood pressure were significantly lower in the ciclosporin-withdrawal group (–3mm Hg and –6mm Hg, respec-

tively) despite significantly less frequent use of antihypertensive medication in this group.

At 2 years, systolic blood pressure remained significantly lower in the ciclosporin-withdrawal group.[68] At 1 year, ciclosporin withdrawal was associated with improved uric acid and magnesium levels. Renal and patient graft survival was not different in the two groups. At 2 years, quality of life was compared in sirolimus-treated kidney transplant patients after ciclosporin elimination and in patients who were still treated with ciclosporin. Of the 361 patients studied, those in the ciclosporin-withdrawal group improved significantly more than others, according to the replies in the 'Kidney Transplant Questionnaire' fatigue and appearance domains. SF-36 vitality score was also better in the ciclosporin-withdrawal group.[69] Regarding the risk of ciclosporin withdrawal in the context of sirolimus treatment in this study, ciclosporin withdrawal with high sirolimus dosage was not associated with any significant effect on patient or graft survival. However, withdrawal was associated with a small but significant increase in the incidence of acute rejection. After randomisation at 3 months post-transplantation, 9.8% of the patients in the withdrawal group experienced a biopsy-proven acute rejection episode versus 4.2% of those who continued on ciclosporin ($p = 0.035$). This increase seems to appear during the early period after ciclosporin withdrawal, especially in patients with low sirolimus trough concentrations. Sirolimus has been associated with other adverse events. Indeed, in the ciclosporin-withdrawal group treated with high-trough sirolimus levels, more thrombocytopenia, hypokalaemia and abnormal liver function test were reported.

Another question is the lipid profile of these patients. After randomisation and high-dose sirolimus, a transient increase in serum triglycerides averaging about 0.4 mmol/L was observed. However, there was no difference between the ciclosporin withdrawal and continuation groups as regards mean concentrations of total cholesterol, calculated low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides. Noteworthy, in the two groups that both continued with sirolimus, 70% of patients were taking HMG-CoA reductase inhibitors and 23% fibrates. In another study,[68] the rates of biopsy-proven acute rejection were not different in the ciclosporin-withdrawal group and continuation group. However, the incidence of acute rejection at 2 months increased from 8.0% to 18.0% at month 6 in the ciclosporin-withdrawal group versus 12.4% to 15.5% in the ciclosporin-continuation group. At 12 months, patient and graft survival was similar in the two groups. Patients in the ciclosporin withdrawal group had higher rates of diarrhoea, abnormal liver test, thrombocytopenia, and hypokalaemia than those in the ciclosporin-continuation group. At 12 months, mean lipid values were slightly higher in the ciclosporin-withdrawal group despite the larger number of patients receiving statins.

In the same study continued after 2 years of follow-up,[68] total cholesterol and high-density lipoprotein cholesterol were still significantly higher in the ciclosporin-withdrawal group. In addition, sirolimus-related adverse effects, such as thombocytopenia, abnormal liver function tests, hypokalaemia, ileus, abnormal wound healing, and infectious pneumonia were more frequent in patients randomised to ciclosporin withdrawal.

In another recent controlled randomised study,[66] 246 renal transplant recipients were enrolled, and 197 of them were randomly assigned to receive either conventional-dosage ciclosporin combined with sirolimus 2 mg/day ($n = 97$) or reduced-dosage ciclosporin with concentration-controlled sirolimus at dosages adjusted to maintain trough concentration levels of 10–20 μg/L ($n = 100$). At month 12, the mean daily dose of sirolimus in the latter group was 6.45mg ± 0.43mg. The reason for the non-randomisation of 49 of 246 patients was the absence of renal function recovery by 48 hours post-surgery. Patients in the ciclosporin-reduced-dose group were eligible for ciclosporin withdrawal if their renal function

was stable at the end of month 2 after transplantation, if they had not been treated for acute rejection in the preceding 3 weeks, and if their sirolimus trough concentrations were 10–20 μg/L. When these conditions were met, ciclosporin was withdrawn completely during the third month after transplantation (25% dose reduction per week for 4 weeks). A total of 82 patients were eligible for ciclosporin elimination. This manoeuvre was successfully completed in 76 patients.

At 12 months, renal function assessed by serum creatinine level and calculated GFR values (Nankivell formula), had improved significantly in the ciclosporin-withdrawal group. This improvement was greater in the patients who had remained rejection-free, but was still significant when those who experienced a rejection were included in the analysis. Unfortunately, specific information regarding renal function in the 'rejector'-group is not available. Hypertension was reported in significantly more cases in the ciclosporin-continuation group. The rates of increased creatinine and hirsutism were higher in the ciclosporin-continuation group. Overall, blood pressure measurements were similar in the two groups. Although at month 12, mean systolic blood pressure was significantly lower in the ciclosporin withdrawal group, mean diastolic blood pressure was similar to that of the ciclosporin-continuation group.

In another study of 30 kidney transplant recipients (22 of 30 with transplants from suboptimal donors) treated initially with a combination of corticosteroids, ciclosporin and sirolimus, patients were randomised to undergo ciclosporin withdrawal or continuation at 3 months.[70] One year after transplantation, renal function, assessed by serum creatinine and creatinine clearance, was significantly better in the ciclosporin-withdrawal group. Interestingly, renal biopsies performed 12 months after transplantation less frequently exhibited severe chronic allograft nephropathy and chronic vascular damage in the ciclosporin withdrawal than continuation group (32% vs 90 and 38% vs 90%, respectively).

5. Ciclosporin Withdrawal and Chronic Allograft Dysfunction

The short-term results of renal transplantation have improved more than the long-term results. The so-called chronic rejection is now termed as chronic allograft nephropathy, in order to take into account the various mechanisms and immunological or non-immunological risk factors, that have been proposed. Among these factors, the importance of the chronic nephrotoxicity caused by calcineurin inhibitors has been stressed. Indeed, the immunosuppressant-induced nephrotoxicity secondary to ciclosporin has become the 'Achilles heel' of immunosuppressive agents.[8]

Mourad et al. reported their long-term experience of ciclosporin reduction (n = 18) or withdrawal (n = 5) in renal transplant recipients with biopsy-proven ciclosporin nephrotoxicity with added azathioprine.[71] After of follow-up of 2 years, renal function had improved and blood pressure was lower after ciclosporin modification. However, with azathioprine, acute rejection was noted in one patient and chronic rejection developed in three patients.

5.1 Ciclosporin Withdrawal with Mycophenolate Mofetil in Unstable Patients

Mycophenolate mofetil was the first of the new immunosuppressive drugs to be used in ciclosporin-withdrawal protocol. This manoeuvre has been tested in a rat model of chronic rejection. In this model, combined treatment with ciclosporin and mycophenolate mofetil did not prevent the development of chronic ciclosporin nephrotoxicity.[72] Nevertheless, ciclosporin withdrawal was associated with improved renal function and histology. Furthermore, mycophenolate mofetil treatment after ciclosporin withdrawal further improved the histological parameters. At the molecular level, mycophenolate mofetil combined with ciclosporin withdrawal was associated with a decrease in the expression of oste-

opontin and TGF-β1 mRNAs.[73] Osteopontin and TGF-β1 have been associated with the development of calcineurin inhibitor-induced nephropathy. Many centres have therefore adopted a policy of either ciclosporin-sparing or stopping regimen in patients with declining kidney function after renal transplantation.

After the preliminary reports by Weir et al.[74] and Ducloux et al.[75] of ciclosporin withdrawal, longer follow-up and larger populations with a control group were studied. Thus, Ducloux et al. have reported the 2-year follow-up of a cohort of 31 renal transplant recipients, whose ciclosporin was completely withdrawn because of chronic allograft nephropathy.[76] In this cohort, serum creatinine level decreased significantly after conversion and remained stable at the end of the follow up. The mean decline in serum creatinine level was –24% after conversion, compared with +20% in the year before conversion. Interestingly, the authors observed a strong inverse relationship between proteinuria at baseline and improvement in renal function. Patients with higher proteinuria were less likely to experience an improvement in their renal function. Another important prognosis factor for renal function improvement after ciclosporin withdrawal was the histology observed at baseline. Isolated ciclosporin nephropathy was associated with the best outcome, whereas renal function improved slightly in patients with Banff grade 1 chronic rejection and remained stable in Banff grade 2 chronic rejection. When ciclosporin was withdrawn in patients with chronic allograft nephropathy, two patients among 18 patients had questionable mild and type II rejection according to the Banff classification. Both received corticosteroids and stabilised. Interestingly, proteinuria significantly increased after 2 years of follow-up in 31 patients. The long-term consequences of this increased proteinuria remain to be determined but must be assessed eventually. In this population, however, even though worsening of histology was present in 4/17 patients, proteinuria was not associated with the worsening of chronic rejection lesions on graft biopsy.

Weir et al. reported the long-term impact of calcineurin inhibitor (ciclosporin and tacrolimus) discontinuation in patients with chronic allograft nephropathy.[77,78] Chronic allograft nephropathy was defined as the chronic deterioration of renal function, assessed by the linear slope of the inverse of serum creatinine over time. All patients underwent allograft biopsies that revealed chronic allograft nephropathy with minimal or no evidence of acute allograft rejection. Among 118 patients, 100 had a reduction of their calcineurin inhibitor and 18 a complete cessation. In the withdrawal group, 91.7% of the calcineurin inhibitor withdrawal group experienced an improvement or no further deterioration in the slope of the reciprocal serum creatinine level after the intervention. This proportion was only 51.7% if the daily dose of ciclosporin was only reduced not withdrawn. Blood pressure was unchanged after ciclosporin withdrawal. There were statistically significant improvements in mean serum cholesterol levels and in mean serum glucose levels after ciclosporin withdrawal. However, Weir and coworkers considered that reduction or withdrawal of calcineurin inhibitors does not help all patients. This is in agreement with the findings from Ducloux et al.[75] that proteinuria and allograft histology at baseline are predictive of the evolution of renal function after conversion.

A large multicentre randomised controlled study was designed to evaluate the effect on graft function of adding mycophenolate mofetil to the immunosuppressive regimen, followed by ciclosporin withdrawal, for patients with biopsy-proven chronic allograft dysfunction.[79] In this study, 143 patients with progressively deteriorating renal function, defined by a negative slope of the reciprocal creatinine level plotted against time were randomised to either ciclosporin withdrawal (n = 73) or continuation (n = 70). In the withdrawal group, renal function stabilised or improved in 58% of the patients compared with 28% in the continuation group. The

follow up of this cohort and the complete evaluation of the baseline characteristics of the patients will be of the utmost importance in improving understanding of the benefits and risks of withdrawal.

5.2 Ciclosporin Withdrawal with Sirolimus in Unstable Patients

Fewer studies have been reported or published concerning the replacement of ciclosporin by sirolimus in patients experiencing chronic allograft nephropathy. The first report came from Dominguez et al.[80] Twenty renal transplant recipients experiencing ciclosporin or tacrolimus toxicity were switched to a fixed daily dose of sirolimus (5mg). In the 12 patients who were switched because of chronic nephrotoxicity, there was a significant 17% decrease in serum creatinine level, which in most cases, remained stable at 12 months. In 7 of 12 patients, renal function improved by >10% (mean decrease of 22%) and in three patients, it remained stable The graft function continued to deteriorate in only two patients. No difference was noted in the initial serum creatinine response to sirolimus in patients with histological signs of chronic rejection or those with nephrotoxicity alone. No significant change in blood pressure was noted.

In another study, 22 renal allograft recipients with biopsy-proven calcineurin inhibitors (ciclosporin and tacrolimus) toxicity were converted 94 ± 63 months after transplantation from a calcineurin-inhibitor to sirolimus.[81] The calcineurin inhibitor dosage was halved at day 1, and then reduced weekly by 10–20% as soon as a therapeutic level was obtained. Serum creatinine level did not improve significantly for up to 6 months after conversion. However, evolution exhibited different patterns as kidney function remained stable in two patients, improved in 11 and further deteriorated in nine. This indicates that a longer follow-up is mandatory. Indeed, a more recent up-date of this cohort was presented at a recent meeting. Among the 24 patients, kidney function had deteriorated further in 13 patients and had improved in 11.[82] Interestingly, the serum creatinine level values of these two groups at conversion were 2.6 ± 0.2 and 3.8 ± 0.5 mg/dL, respectively. One explanation for the absence of improvement in some patients may be the severity of renal dysfunction before the calcineurin inhibitor withdrawal. No acute rejection episodes occurred. Sirolimus therapy was discontinued in six patients because of haemodialysis (n = 3), bad taste of the sirolimus solution (n = 2) and recurrent nephritic syndrome after withdrawal of ciclosporin (n = 1).

This latter complication has also been reported by Peraldi et al.[83] After late withdrawal, 8 of 28 patients in their cohort developed nephrotic syndrome, with focal segmental glomerular sclerosis on the allograft biopsy in six cases.[83] The other main adverse events were anaemia, epistaxis, thrombocytopenia and leukopenia. No significant change was noted in the lipid profile, but five patients required HMG CoA reductase inhibitor therapy initiation or an increase in their previous HMG CoA reductase inhibitor dosage. Targeted trough levels may be important for the development of some adverse events. Thus in the same population, Peraldi et al. reported pneumonitis with trough levels of 20 µg/L.[83] This rare complication of sirolimus has indeed occurred more often in long-term renal transplant recipients who were switched from ciclosporin to sirolimus.[84,85] Interestingly, in the initial report by Dominguez et al., 5 of 17 patients developed pneumonia, *Pneumocystis carinii* pneumonia in two cases and bronchiolitis obliterans in two.[80] Of the 18 patients switched to sirolimus, pneumonia developed in five patients and oral aphtous ulcer in nine. There was no acute rejection in any of long-term patients reported. All patients had a transient decrease in their platelet count, but these counts returned to the pre-switch level within 1 month. Serum cholesterol and triglyceride levels rose by an average of 15% in half the patients after the switch. By 1 year, the triglyceride levels were back at baseline in all but five patients. After lipid-lowering medication, cholesterol levels improved.

6. Discussion and Conclusions

Although ciclosporin has been associated with a constant improvement in the results of renal transplantation,[86] its use is also responsible for various short- and long-term toxicities, including nephrotoxicity, and for the worsening of cardiovascular risk factors.[29] Studies have shown that when ciclosporin can be successfully withdrawn, there is a significant improvement in renal function, hypertension and lipid levels. Another potential benefit might be a lower risk of long-term cancer, because low-dose ciclosporin has already been linked to the development of carcinologic complications.[87]

Ciclosporin withdrawal is also associated with a sustained increase in the risk of acute rejection episodes, but its effect on long-term patient and graft survival are not known except during the azathioprine era.[48] It is therefore necessary to determine the best treatment that should be combined with ciclosporin withdrawal, and to define the population of patients with an acceptable benefit-risk balance.

When used with azathioprine, ciclosporin withdrawal is associated with a significant increase in acute rejection episodes.[47,48] Consequently, because ciclosporin withdrawal has been shown to be more feasible for patients treated with mycophenolate mofetil than azathioprine,[58] the first thing to do when ciclosporin withdrawal is desired is to switch azathioprine to mycophenolate mofetil.

If the patient is already being treated with an immunosuppressive drug, such as mycophenolate mofetil or sirolimus, this treatment should be continued. These new treatments have provided an opportunity to re-assess the effects of various immunosuppressive drugs together with ciclosporin withdrawal. Sirolimus seems to be associated with better prevention of acute rejection episodes since the increase of the incidence of such rejection when ciclosporin was withdrawn 3 months after transplantation was not statistically significant, although cardiovascular risk factors, especially hyperlipidaemia, developed in most patients.[65] Conversely, ciclosporin withdrawal from mycophenolate mofetil-treated patients was associated with a small but significant increase in the incidence of acute rejection episodes, but also with a very good safety profile.[61] To identify the regimen that achieves the most beneficial metabolic outcomes, it is clear that prospective, randomised studies in which sirolimus + corticosteroids are compared with mycophenolate mofetil + corticosteroids as maintenance therapy, are of major importance for renal transplantation. Another approach should be to combine sirolimus and mycophenolate mofetil after ciclosporin withdrawal, to obtain a non-nephrotoxic maintenance treatment.

The next problem is to define the population of patients that can benefit from ciclosporin withdrawal. This is particularly necessary for stable patients since there is no clear indication, like chronic allograft deterioration, that makes such withdrawal mandatory. Clinical trials of sirolimus[65] or mycophenolate mofetil[61] have failed to define the elective population for ciclosporin withdrawal. It is noteworthy that in the sirolimus trial, only 82% of the patients were eligible for ciclosporin withdrawal 3 months after transplantation. In both the mycophenolate mofetil and the sirolimus trials, ciclosporin was effectively withdrawn from 93% and 97% of patients, respectively.

Anjum et al. recently reported an attempt to define the risks of withdrawal in stable kidney transplant recipients.[54] Even though their study was monocentric, one interesting item of information given by the authors was their the definition of various populations. They reviewed the records of each of the 996 transplant recipients included and classified them according to the nature of their ciclosporin regimen, as follows: (i) total absence of the use of ciclosporin for patients considered to be at low risk of acute rejection (n = 156); or (ii) use of ciclosporin, but without addressing the question of its withdrawal for patients who failed to survive for at least 1 year with a functioning kidney (n = 93). After exclusion of these two populations, the remaining 747 patients were considered for ciclosporin withdrawal, and assigned to following

groups: (i) ciclosporin discontinuation due to ciclosporin toxicity (n = 41, 5.5%); (ii) ciclosporin continuation due to multiple, and/or severe acute rejections (n = 76, 10.1%); (iii) ciclosporin elective withdrawal after the first year (n = 464, 62.1%); and (iv) ciclosporin elective continuation (n = 166, 22.2%). To define the risks of ciclosporin withdrawal, only patients in the last two categories were included in the analysis (n = 630). For those undergoing elective ciclosporin withdrawal, its tapering began at 1.35 ± 0.69 years after transplantation, and follow-up lasted for 5.89 ± 3.52 years after the beginning of ciclosporin withdrawal. Acute rejection episodes within 6 months after starting elective ciclosporin withdrawal occurred in 7.3% of patients between 1986–1989, but declined to 4.5% after 1989. The best predictor of acute rejection was the number of HLA-DR mismatches. The incidences of acute rejection within 6 months were 3.9, 4.5, and 13.3% for 0-, 1- and 2-HLA-DR mismatches, respectively. Risk factors included the transplant era, age at transplantation, obesity and number of HLA-B and DR mismatches. In this analysis, the risk of acute rejection was also similar in patients treated with mycophenolate mofetil (4 of 67, 5.6%) and those treated with azathioprine (26 of 314, 7.7%). However, the number of patients included in the analysis is too small to allow firm conclusions to be drawn.

For stable patients, the best therapeutic option may be ciclosporin-sparing rather than complete withdrawal, at least until more information is available about the efficacy of a sirolimus-mycophenolate mofetil-corticosteroid association after ciclosporin withdrawal. Gradual calcineurin inhibitor decrease is also a potential alternative minimisation strategy for stable renal transplant patients.[88,89] In these studies, immunosuppression regimen has included mycophenolate mofetil, daclizumab and corticosteroids and there was with no increase in the frequency of infectious complications. Preliminary results suggest that the ciclosporin dose can be halved without causing complications. The overall rate of rejection episodes was not affected. Renal function also displayed significant improvement. In another large monocentric trial during the azathioprine era, ciclosporin reduction was associated with the absence of a negative impact on renal allograft survival, but with a significant reduction of neoplasia development.[87] The largest improvement in serum creatinine level probably occurred after the reduction of ciclosporin to one-third of its baseline value. The timing and extent of changes in serum lipid levels exhibited a similar pattern. This pattern, together with the timing of the onset of acute rejection episodes, suggests that only the reduction of ciclosporin dosage is necessary and safe, and may constitute a valid alternative to withdrawal. However, recent data showed that low doses of ciclosporin may still promote interstitial fibrosis.[27]

As regards patients whose clinical condition is unstable, little information exists concerning ciclosporin withdrawal combined with sirolimus treatment. This strategy is currently being explored in a prospective trial. More data are available for ciclosporin withdrawal in patients treated with mycophenolate mofetil. Ducloux et al. provided important information in this respect,[76] by showing that both allograft histology and proteinuria at baseline are predictive of the renal function after conversion. Before any ciclosporin withdrawal, renal biopsy should be performed to demonstrate chronic ciclosporin nephrotoxicity. For patients exhibiting such toxicity, ciclosporin withdrawal is indicated. A prospective trial in this situation will be soon be completed.[79] It includes those with chronic allograft dysfunction characterised by gradually rising serum creatinine levels. A preliminary report indicated that the substitution of mycophenolate mofetil for ciclosporin was safe, and halted deteriorating renal function in 58% of patients.

Finally, besides ciclosporin withdrawal, there is increased interest in the design and implementation of avoidance protocols, i.e. immunosuppressive regimens designed to avoid treatment with ciclosporin or eliminate it completely. The major disadvantage

of ciclosporin sparing or withdrawal is that patients must be exposed to ciclosporin in the first place. There is evidence that ciclosporin-associated renal toxicity may occur early, and progress in the native kidney of pancreas and lung transplant recipients and in patients with uveitis, even after dose reductions.[9,20,27] Another concern is the effect of calcineurin inhibitors on the induction of immune tolerance. Calcineurin inhibitors have been shown to limit T-cell activation by blocking susceptibility to apoptosis and activation-induced cell death.[90-94] Many preliminary studies of ciclosporin avoidance have been reported. One such report gave the results of an immunosuppressive protocol combining daclizumab as anti-CD25 monoclonal antibody, mycophenolate mofetil and corticosteroids.[95] The incidence of acute rejection was 45% in a population of 98 renal transplant recipients. Results have also been published for the combination of corticosteroids, sirolimus and either azathioprine or mycophenolate mofetil in two studies,[96,97] which concluded a rather high incidence of acute rejection episodes (41 and 27.5%, respectively) as well as different toxicity profile from that observed with ciclosporin. Lastly, two recent reports may have pointed the way to efficient new approaches. Flechner et al. have administered a combination of basiliximab, a chimeric anti-IL2 receptor monoclonal antibody together with mycophenolate mofetil, corticosteroids and sirolimus,[98] a regimen that provided comparable 1-year patient and graft survival. In addition, at 1 year the incidence of acute rejection episodes reaches an impressive 6.4% compared with 16.6% in the controls on a ciclosporin-based regimen. Renal function, measured by the creatinine clearance, was also significantly better in the ciclosporin-free group (81.1 vs 61.1 mL/min, respectively) and did not tend to worsen during the first year after transplantation. The initial results from John Swanson[99] are even more striking, as these authors reported the positive results for kidney transplantation with rabbit antithymocyte globulin induction associated with sirolimus monotherapy.

In conclusion, ciclosporin withdrawal now seems feasible, thanks to the use of new immunosuppressive drugs. In stable patients, however, it may not be superior to ciclosporin sparing. The best definition of the population of patients who can benefit from withdrawal is still a matter of debate. In unstable patients, ciclosporin withdrawal is usually safe if ciclosporin-nephrotoxicity is part of the mechanism involved in chronic allograft dysfunction. In these patients, such withdrawal is usually safe and associated with significant improvements in both renal function and cardiovascular risk factors, especially when a mycophenolate mofetil-based therapy is used. Finally, protocols designed to explore the effects of complete avoidance of calcineurin-inhibitors and of a truly non-nephrotoxic regimen are due to be published in the near future. Investigations of the impact of improved renal function on graft survival, histological evidence of graft integrity, and chronic rejection rates requires longer follow-up periods than those so far reported. In any case, not all patients could benefit from these new approaches and new concepts, which more than ever illustrate the need for immunological and non-immunological markers to enable immunosuppression to be tailored to the requirements of specific patient populations.

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