

# Long-Term Immunosuppression in Pediatric Liver Transplantation

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## Abstract

*Pediatric liver transplantation is a successful treatment with prolonged survival in 80-90% of patients. Immunosuppression has a crucial role in allowing graft survival. The importance of an appropriate balance between protection from rejection and avoidance of the side effects of immunosuppressive drugs is universally recognized. Most trials have been focused on minimizing acute rejection in the early postoperative period. The management of immunosuppression in the long term has been a subject of general description with few detailed studies or trials.*

*Long-term immunosuppression in pediatric liver transplant patients consists of a calcineurin inhibitor at lower blood levels compared to the target in the early posttransplant period, to which low-dose steroids or mycophenolate may be added. Nearly 30% of patients exhibit graft dysfunction of various causes. Once a biliary problem is ruled out, the reasons for dysfunction are rejection, autoimmune hepatitis, or idiopathic problems in which an immunologic basis is highly suspected. Most of these cases are detected in the subclinical stages, and are managed with increased immunosuppression. Noncompliance complicates the evolution of the graft in adolescents and young adults. Altogether, very few patients lose the graft in the long term; however, protocol biopsies indicate a high rate of abnormal histology in contrast to normal biochemistry.*

*Published information on immunosuppression in the long term, the choice of drugs, drug monitoring and methods to evaluate compliance, immune-related causes of graft dysfunction, and attitudes to renal function sparing have been reviewed.*

*Tacrolimus and cyclosporine are both safe options for primary immunosuppression, but tacrolimus is preferred by most centers because of the reduced risk of refractory rejection and cosmetic benefits. Eighty percent of the patients do not need steroids, but no trial has adequately compared the benefit/risk ratio of steroid maintenance at low doses, still used in many centers. Mycophenolate allows decreasing the calcineurin inhibitor level and is applied for renal sparing in selected children, showing a decrease in glomerular filtration rate, which can be detected early by measuring cystatin C levels. Blood levels are the current method for drug monitoring. Some biomarkers of immune cell function are starting clinical*

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**use. An excessive variation of blood levels is the best marker of noncompliance, which affects up to 40% of patients in adolescence.**

**The incidence of late acute rejection is 10%. It is a difficult diagnosis in the long term even after a panel of experts' revision of criteria. No gross differences in incidence or outcome are seen in centers with different immunosuppression protocols. Chronic rejection and autoimmune hepatitis occur in 5-10% overall. Long-term liver pathology indicates lesions resembling chronic hepatitis or early stages of chronic rejection that are observed in many cases in contrast to minor biochemical abnormalities or normal liver function.**

**Current immunosuppression practices achieve equivalent numbers of patients with relevant conditions related to under- or over-immunosuppression, but should be the subject of more investigations for the correct management of late graft dysfunction and biochemically silent graft damage. (Trends in Transplant. 2009;1:28-34)**

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## Key words

**Liver transplantation. Children. Long-term follow-up. Graft function. Immunosuppression.**

## Introduction

Liver transplantation has been applied for children for more than two decades, with increasing patient and graft survival rates. The number of children undergoing liver transplantation in Europe in the period 1988-2006 was 6,089 (European Liver Transplant Registry, September 2007); a total of 5,675 children, approximately 600 per year, underwent liver transplantation in the USA and Canada from 1996 to 2005<sup>1</sup>. Current patient survival approximates 90% at year 10 in the main centers of Europe, Japan, and the USA. The USA database from 1994 to 2006 shows that actuarial graft survival was 84.0% at one year and 77.3% at four years; patient survival at four years was 85.5%<sup>2</sup>. The improved patient and graft survival is attributed to advances in surgery and improved immunosuppression regimens.

Immunosuppression has evolved over time subject to two caveats: the availability of cyclosporine (cyclosporin A, CsA) and of tacrolimus (TAC). Additional drugs designed for intense acute immunosuppression (rabbit antithymocyte globulin, anti-CD25, anti-CD52) or for chronic use (mycophenolate, mTOR inhibitors) have become available as well. Different combinations are possible, with a calcineurin inhibitor (CNI) as main therapy.

The best immunosuppression regimen is the one allowing a balanced risk of rejection and adverse effects. Early and late postoperative periods have special features, and priorities change from avoiding rejection in the first period to maintaining the patient with minimal toxicities and infection in the long term.

## Primary immunosuppression

### Calcineurin inhibitors

Only one trial is available to compare tacrolimus and cyclosporin microemulsion as primary immunosuppression in children<sup>3</sup>. The study was multicentre: 181 patients were recruited, 91 children received TAC (target level 10-15 ng/ml) and 90 received cyclosporine (CsA), combined with steroids and, in the cyclosporine arm only, associated to azathioprine.

The 12-month results were published in 2004<sup>3</sup>. Patient and graft survival were equally high in both groups (patient: 93.4% TAC vs. 92.2% CsA; graft: 92.3% TAC vs. 85.4% CsA). Tacrolimus allowed higher rejection-free survival, with a significantly lower risk of steroid-resistant rejection (5.5% TAC vs. 26.7% CsA;  $p = 0.0001$ ). Bacterial, fungal, and cytomegalovirus infections affected nearly the same number of

children in both arms; no differences were noticed in the average decrease of glomerular filtration rate (GFR).

A long-term analysis was done with 146 patients (74 TAC, 72 CsA) participating in the above study<sup>4</sup>. Two to five years after liver transplantation, there were 97 patients still receiving the drug to which they were originally randomized (59 TAC, 38 CsA), and 49 patients had been withdrawn from receiving it for various reasons (15 TAC, 34 CsA). A lower risk of chronic rejection was observed in the TAC group (completers: 1/59 TAC vs. 6/38 CsA; withdrawn: 0/15 TAC vs. 2/34 CsA). The incidence of posttransplant lymphoproliferative disease was 3/74 TAC and 5/72 CsA. Five years after randomization, 78% of children in the TAC arm and only 33% in the CsA arm maintained the original CNI. Patient survival up to nine years after transplantation remained similar according to the initial CNI randomization. The conclusion of the study indicates that TAC is more effective in preventing acute and chronic rejection with fewer adverse effects.

Both CsA and TAC offer equivalent rates of patient survival and are safe alternatives for children undergoing liver transplantation. However, the higher efficacy of TAC explains the change over the past 10 years towards a TAC-based primary immunosuppression in most centers. Data from the USA show that in the last five years, around 90% of pediatric liver transplant recipients received a TAC-based maintenance immunosuppression therapy; CsA use has decreased from 22% of patients to only 4% in 2005<sup>1</sup>.

The relative risk of rejection is 1.49 in children on primary immunosuppression with CsA compared to those on TAC, but episodes of rejection in the first six months are not predictors of graft failure<sup>2</sup>. Chronic rejection justifies only 14% of the retransplantation procedures in children<sup>5</sup>. These facts are arguments for avoiding aggressive primary immunosuppression in the early posttransplant period, as infection causes more morbidity than rejection and is the main cause of mortality.

Chronic rejection is rare, both in children on TAC and in patients who receive CsA but are changed to TAC in the case of steroid-resistant rejection. A German series observed that 19% of the CsA-treated patients needed conversion to TAC<sup>6</sup>.

The reasons for TAC preference in the long-term follow-up are the exceptionality of chronic rejection, unchanged physical appearance, and the avoidance of side effects related to CsA (hirsutism, gum hyperplasia). The main reason for conversion from TAC to CsA is the appearance of food allergy, a problem presented in 10% of children who underwent liver transplantation at a very young age; food allergy usually disappears on CsA treatment<sup>7</sup>.

## **Steroids**

Steroid-free protocols are frequently discussed; however, 84% of pediatric liver transplant recipients in the USA were discharged on maintenance corticosteroids in 2005<sup>2</sup>. Most European centers apply steroids in the early period after liver transplantation.

Nearly 50% of European and USA centers do seek steroid-free regimens, starting between 3-12 months posttransplantation. In the experience of Pittsburgh, 98.5% of children on TAC could be weaned off steroids; only 22% needed reinstatement of steroids for rejection or renal dysfunction<sup>8</sup>. Using primary immunosuppression with CsA, most children at Hamburg were off steroids in the follow-up<sup>6</sup>.

Maintenance steroids at low doses (usually on alternate-day basis) do not apparently make differences in growth compared to steroid-free regimens. The anti-inflammatory properties of steroids could be of value in preventing graft damage in the long term.

## **Monitoring immunosuppressive treatment**

Blood levels of CsA or TAC are determined to assess immunosuppression. Levels are checked every 2-3 months in stable patients. Maintenance at a level of TAC 4-6 ng/ml or trough CsA 80-120 ng/ml is common beyond one year. Trough CsA blood level has been substituted by two hours post-dose (C<sub>2</sub>) levels in many centers because of the better correlation to drug exposure. Trough mycophenolic acid levels ranging from 1.5-3 mg/l are adequate for liver transplanted children receiving mycophenolate mofetil (MMF) in association to a CNI<sup>9</sup>. Mycophenolic acid blood levels do not correlate to the area under the curve and

novel methods to guide MMF dosing are proposed with the measurement of inosine monophosphate dehydrogenase activity<sup>10</sup>.

The usual practice of assessing drug trough levels may not reflect overall immune suppression. Some assays have been developed to estimate immune response such as the measurement of soluble CD30, nuclear factor of activated T-cell-regulated gene expression, profiles of circulating cytokines, and circulating regulatory T-cells, but these have not translated into clinical value<sup>11,12</sup>.

The immune cell function assay (ImmuKnow®, ViraCor Laboratories, USA) for assessment of cell-mediated immunity in an immunosuppressed population is designed to measure increases in intracellular adenosine triphosphate (ATP) of CD4 T-cells following activation by the mitogen PHA (phytohemagglutinin). The ImmuKnow assay is an additional tool in transplant patient management, but probably it evaluates the effect of steroids and CNI, and not that derived from mammalian target of rapamycin (mTOR) inhibitors or MMF. In adults, the degree of immune function as assessed by the ImmuKnow assay helps to predict patients at risk for infection or rejection. Trials have compared immune responses in healthy adults and stable transplant recipients so that three zones of immune response were established: strong ( $\geq 525$  ng/ml ATP), moderate (226-524 ng/ml ATP) and low ( $\leq 225$  ng/ml ATP). In adult patients, low ATP values ( $< 25$  ng/ml) predispose to infection (12-fold) and high ATP ( $> 700$ ) increased the odds of rejection 30-fold<sup>13</sup>. The study in children revealed that healthy children ( $< 12$  years) had statistically significantly lower immune function values than healthy adults, and that pediatric renal transplant recipients were more immunosuppressed than adult transplant recipients. The adjusted zones for children under 12 years are: strong  $\geq 395$ , moderate 176-394, and low  $\leq 175$  ng/ml ATP<sup>14</sup>.

The ImmuKnow assay has been applied in the evaluation of liver transplanted children with Epstein-Barr virus (EBV) infection. Patients with low EBV loads had a significantly ( $p < 0.04$ ) stronger immune response to PHA than patients with EBV load  $> 1,000$  copies/ $\mu$ g DNA. All patients with ATP  $< 125$  ng/ml showed a high EBV load ( $> 4,000$  copies/ $\mu$ g DNA). When immunosuppression was reduced, an increase of the ATP release was observed that correlated with a decrease of the EBV viral load<sup>15</sup>.

## **Nonadherence to the immunosuppressive regimen**

In the pediatric transplant setting, it is common to encounter adolescent patients who take their medications with admitted accidentally omitted doses, but the extent of missed doses is usually difficult to assess. Nonadherence to medication is significantly associated to late acute rejection. In a series of 111 patients 12-21 years old, 45% were identified as nonadherent, defined by at least one episode of admission by the patient of not taking immunosuppressive medications or not attending any clinical visit in a retrospective review of a one-year period. Among 30 cases with sporadic or complete discontinuation of drugs, late acute rejection occurred in 33%, compared to 9.3% in adherent patients<sup>16</sup>.

In adolescents and young adults, measuring adherence is crucial to maintain graft function, allowing earlier psychosocial and behavioral interventions. Several studies concluded that the degree of fluctuation of levels of TAC over successive outpatient visits was the best measure of adherence. A standard deviation (SD) higher than 2, 2.5, or 3 ng/ml is an indicator of noncompliance. Dose modifications made by the physician may also contribute to variations of TAC levels. A study was taken to minimize that confounder by standardizing physician practice patterns in adjusting TAC dosing, and by recognizing many explainable reasons for variations of levels so that dose was not modified routinely. Over the period of the study, an increasing proportion of patients (initial 55%, evolutive 75-85%) had their TAC levels within the therapeutic range, which was interpreted as the effect of improved compliance by patients who had been informed about the program of vigilance of levels in order to assess adherence to treatment. Eleven episodes of late acute rejection occurred during the study period of one year in 101 patients; 10 of the 11 episodes occurred in patients who had TAC level SD  $> 2$ . The incidence of rejection was 1% in patients with SD  $< 2$ , while 28% of patients with TAC SD  $> 2$ , and 67% of those with SD  $> 3$  developed acute rejection. Good compliance and not a higher mean TAC level influenced the risk of rejection, as only 2% of 50 children with mean TAC blood levels  $< 5.27$  ng/ml had rejection, compared to 18% in the remaining 51 patients with mean values  $> 5.27$  ng/ml<sup>17</sup>.

Other investigators found the cutoff value of SD  $\geq 2.5$  in TAC values was useful to guide clinical

cians, as patients had about eight-times higher odds to develop rejection, and it provided a satisfactory balance to differentiate true nonadherence and the variations of levels attributable to changes in absorption, body mass, intervening illness, and drug interactions<sup>18</sup>.

Children with chronic disease often experience a delay in personal maturation and independence<sup>19</sup>. Chronologic age alone is not an adequate guide to initiate the full responsibility in taking immunosuppressive drugs. The essence of timing of all aspects of transition is that of flexibility, timing of events in the transitional process of a young patient taking a primary role in the medical consultation until transition to adult services must be individualized and planned years beforehand<sup>20</sup>.

Difficulties of adherence in late adolescence can deteriorate further with the transition from pediatric care to adult services. A short series of 14 patients showed median TAC SD was 3.2 before transition, but 4.08 and 5.09 in the first and second years, respectively, under follow-up in adult clinics<sup>21</sup>. Adherence was significantly poorer for transitioned patients than for a cohort of adolescent patients still handled by pediatricians. Proposals to manage the problem include "teen clinics", or a delay of transition to adult services until the age of risk is overcome.

### **Rejection and other immune-mediated causes of graft damage in the long term**

Most late causes of liver allograft injury are first detected because of abnormalities in routinely monitored liver tests; clinical signs and symptoms are much less common.

The problem, usual in adult patients, of differentiating recurrence of pretransplantation disease is limited to very few children who needed transplantation for viral or autoimmune hepatitis. Most commonly, biliary disease due to anastomotic strictures, or intrahepatic strictures secondary to previous ischemic damage constitute the main conditions to be ruled out along with late rejection. Posttransplant *de novo* autoimmune hepatitis should be included in the differential diagnosis of pediatric liver transplant patients without previous autoimmune liver disease who develop late graft dysfunction.

## **Rejection**

The definite diagnosis of rejection can be difficult when facing liver allograft dysfunction occurring more than one year after transplantation. Histopathologic features of late rejection are somewhat different from acute rejection occurring early after transplantation. The Banff Working Group on Liver Allograft Pathology described late acute rejection as having fewer blastic lymphocytes, slightly greater interface activity, less venous subendothelial inflammation, and slightly more lobular activity. It can also present as isolated perivenular inflammation and hepatocyte dropout (so-called "central perivenulitis") and evolve into typical chronic rejection with ductopenia. Subendothelial inflammation of portal or central veins is not a required finding in such cases. Late acute rejection, however, is still most commonly characterized by predominantly mononuclear portal inflammation containing lymphocytes, neutrophils and eosinophils, venous subendothelial inflammation of portal or central veins or perivenular inflammation, and inflammatory bile duct damage<sup>22</sup>.

Children followed in the long term develop a 10% annual rate of biopsy proven rejection or a liver dysfunction with nonspecific histologic changes that ultimately receives treatment as a rejection episode<sup>17,23-25</sup>.

A cumulative rate of chronic rejection occurs in 6% of children followed in the long term<sup>26</sup>. Most cases are thought to be related to nonadherence to immunosuppressive treatment.

## **Autoimmune hepatitis**

Posttransplant *de novo* autoimmune hepatitis (d-AIH) is increasingly described as a long-term complication after pediatric liver transplantation. It is characterized by graft dysfunction, the development of autoimmune antibodies, and histologic evidence of hepatitis in liver transplant recipients without a previous history of autoimmune liver disease. This disorder affects 2.1-5.2% of pediatric liver transplanted patients.

It remains unclear whether d-AIH represents an autoimmune condition or a form of chronic rejection. Forty-one of 619 patients in the UCLA series were ultimately identified as having hepatitis-AIH (incidence, 6.6%). The median duration be-

tween transplantation and the development of d-AIH was 6.7 years. Specific differences at the time of diagnosis revealed that prior to the diagnosis of d-AIH, patients had more episodes of rejection, an increased dependence on steroids, and overall greater immunosuppression requirements than their matched controls. Fewer of the cases (46%) versus controls (81%) were off prednisone, fewer of the cases (29%) versus controls (63%) were on monotherapy CsA or TAC, and a trend was noted that most of the cases (39%) versus controls (24%) required MMF or azathioprine as maintenance medications. Additionally, there was a statistically significant difference in the mean TAC levels at diagnosis of the d-AIH patients ( $7.1 \pm 1.2$  ng/ml) relative to the controls ( $5.3 \pm 1.0$  ng/ml)<sup>27</sup>.

### **Idiopathic graft damage**

A substantial proportion of children show inflammatory liver lesions that usually go unrecognized by a normal biochemistry. Protocol biopsies were performed in a series of 158 children<sup>28</sup>. Normal or near-normal histology was reported in 77 of 113 (68%), 61 of 135 (45%), and 20 of 64 (31%) at one, five, and ten years, respectively. The commonest histologic abnormality was chronic hepatitis, the incidence of which increased with time: 22, 43, and 64% at one, five, and ten years, respectively. The incidence of fibrosis associated with chronic hepatitis increased with time: 52, 81, and 91% at one, five, and ten years, respectively. Aspartate aminotransferase (AST) levels were slightly elevated in children with chronic hepatitis (median levels 52, 63, and 48 IU/l at one, five, and ten years, respectively), but this did not reach statistical significance compared with those with normal histology. The most important factor associated with chronic hepatitis was the presence of autoantibodies, noted in 72 and 80% of cases at five and ten years, respectively, compared with 13 and 10% of cases with normal or near-normal histology. However, only four children with chronic hepatitis and autoantibodies had other features supporting a diagnosis of *de novo* AIH. Chronic hepatitis may represent a form of chronic rejection related to under-immunosuppression. Most of the children in that study received CsA as monotherapy from one year posttransplantation, and steroids were usually withdrawn at three months<sup>28</sup>.

As pointed out in that study, the prevalence of autoantibodies in pediatric liver recipients is

high in the long term. In the series of Hamburg, positive markers were detected in 74%, while liver dysfunction was observed in 46% of these children (none had AIH) and in 35% of the children seronegative for autoantibodies<sup>29</sup>. In an Italian series, 24% of patients had positive autoantibodies, of whom 37% suffered graft disease with either early chronic rejection (perivenular drop-out and venulitis without ductopenia) or autoimmune hepatitis. Autoimmune hepatitis improves with conventional steroid plus azathioprine treatment<sup>26</sup>.

### **Renal-sparing immunosuppression regimens**

The monitoring of renal function and blood pressure is a key part of post-liver transplant care. Calcineurin inhibitor-induced acute and chronic arterial vasoconstriction mediates nephrotoxicity leading to a decrease in GFR and tubular damage. Severe renal insufficiency develops in 5% of liver transplant patients in the long term<sup>30</sup>. The proportion of children with significant renal dysfunction rises to 30%. A median 30% fall in GFR is observed in the long term compared to pretransplant values in children on TAC or CsA<sup>31</sup>

Creatinine-based estimates are not very sensitive to moderate decreases of GFR, and early recognition of CNI-induced nephropathy is important since safe, alternative immunosuppressive regimens can be applied. Cystatin C  $> 1.06$  mg/l is an easy and reliable index of <sup>51</sup>Chromium-ethylenediamine tetraacetic acid GFR  $< 80$  ml/min, a reasonable threshold for actions towards prevention of further deterioration<sup>32</sup>.

The method for sparing renal function has consisted of CNI reduction (usually plus azathioprine/MMF)<sup>33</sup>. Reduced doses of CNI associated to MMF led to improvement in renal function parameters in 82% of cases with renal dysfunction secondary to the prolonged use of CsA or TAC, and none experienced rejection<sup>34</sup>. The dosage of MMF required is lower in children on TAC compared to those on CsA<sup>35</sup>. In a short series of children who had received a liver transplant more than six months prior the study, a 12-hour pharmacokinetic profile showed, when used in combination with CsA, a MMF dose of 740 mg/m<sup>2</sup> twice-daily would be recommended in pediatric liver transplant recipients to achieve mycophenolic acid exposures similar to those observed in adult liver transplant recipients<sup>36</sup>.

A CNI-devoid regimen (transfer to MMF or sirolimus) has been experienced in selected patients. Transfer to MMF (20-40 mg/kg/day) in 48 children (median 4 years posttransplantation) significantly improved the calculated GFR from 54 to 77 ml/min/1.73 m<sup>2</sup> (median at baseline and second month, respectively). Beneficial effect with recovery was seen in a group, while patients with end-stage renal failure at baseline just sustained calculated GFR values. Since 14% of patients experienced liver function test abnormalities, association of steroids for an initial three-month period was recommended<sup>37</sup>.

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