CLINICAL INVESTIGATION

A review of the long-term outlook of children and young people post liver transplant

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The spectrum of pediatric liver disease is wide, encompassing a number of causes both acute and chronic including metabolic, infective, malignant, drug-induced, autoimmune and idiopathic. Liver transplant is life changing. It is a successful treatment for end-stage liver disease and has been widely available in the UK as a treatment option for over 30 years. Prior to this, patients inevitably succumbed to their underlying condition. Early survival rates post-transplant were low (28%) due to the complications encountered, including rejection due to inadequate immunosuppression, biliary and vascular complications. There have been many advances in management, including development of our understanding of immunosuppression, as well as better medical and surgical management in the peritransplant period. Innovative surgical techniques such as reduced or split adult donor grafts or living donor grafts have transformed the ability to transplant pediatric recipients who rarely survive long enough to obtain a whole liver.

Keywords: immunosuppression • liver transplant • nonadherence • pediatric • survival • transition

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The outcome of liver disease in the pediatric population

As immediate survival improves, we can now focus on the long-term outcomes, which we define as being more than 5 years post liver transplant. There are few studies looking at the long-term outcome of pediatric patients post-transplant, and of those that have been published, the majority focus on the long-term complications associated with the procedure such as rejection (acute and chronic), renal impairment, development of hematological complications and malignancies. The aim of this review, therefore, is to present the most recent information available focusing on changes in the management in the peri- and post-transplant period, including immunosuppression, prevention of side effects, transition in later childhood and advice on long-term follow up.

Liver disease in the pediatric population

The single most common cause of chronic liver disease and liver failure in the pediatric population is cholestatic liver disease, namely extrahepatic biliary atresia (Figure 1A). This developmental defect is treated with palliative surgery, the Kasai portoenterostomy in early infancy, but there is a long-term risk of developing chronic liver disease and the eventual need for liver transplantation.

Liver transplantation has been a major development in the treatment of liver disease and has changed the lives of those living with it. There are on average 3000 liver transplants in Europe every year. Survival rates have improved as surgical techniques and immunosuppression protocols are modified and enhanced. Current estimates of 1-year survival in the pediatric population can be as high as >92% following liver

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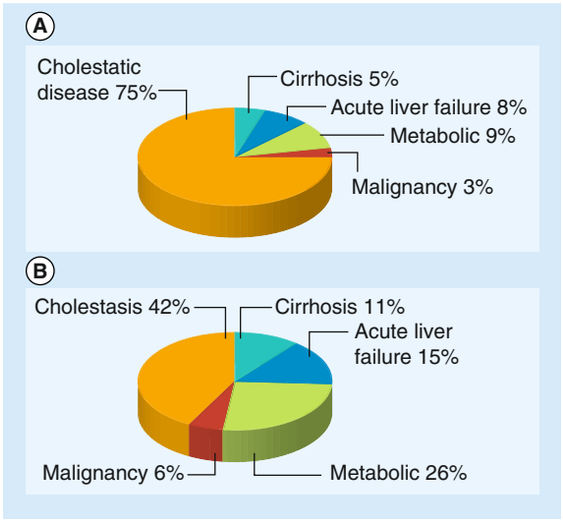

Figure 1. Indications for liver transplantation.
(A) Indication for liver transplantation in children aged 0–2 years (n = 3700). **(B)** Indication for liver transplantation in the 2–15 years old age group (n = 4729.
Data taken from European Liver Transplant Registry, relating to children transplanted from 1968–2011 [1].

transplantation [1]. This compares favorably to early transplants where the recipient was at risk of multiorgan failure, overwhelming sepsis, poor graft function and acute severe rejection. Inevitably survival rates were far lower. Even with the advent of newer techniques and regimes, the risk of renal impairment resulting in death or dialysis remains a substantial concern. Abnormalities of carbohydrate and lipid metabolism as a result of these immunosuppressant regimens can also have a long-term cardiovascular risk.

Liver transplantation has dramatically improved the long-term prognosis for many children dying of end-stage liver disease. Elements responsible for improving patient survival include:

- Better preoperative management of biliary/vascular complications and nutritional support;
- The use of split/reduced/living related donor grafts, which ultimately expands the potential organ pool;
- Evolving immunosuppression.

In chronic liver disease, the indications for transplant relate to the underlying diagnosis (Figure 1). In biliary atresia for instance, urgent transplantation is indicated in children with failed Kasai portoenterostomy and nutritional or hepatic complication, such as reversed vessel blood flow, ascites, recurrent cholangitis, development of cirrhosis and portal hypertension, development of malnutrition or growth failure despite adequate nutritional support, such as nasogastric feeding. The British Pediatric Surveillance Unit review by McKiernan *et al.* presented the data of a 13-year review of children undergoing Kasai portoenterostomy for biliary atresia. In total, 91 children were identified as having undergone Kasai portoenterostomy in 15 UK centers. However, of these 15 centers, only two performed more than five Kasai procedures per year. A total of 15 children (16%) died: two out of three following unsuccessful portoenterostomy, one of sepsis after successful portoenterostomy, and four after liver transplantation. Nearly half (42 children) underwent liver transplantation. The median age was 1 year (range 0.5–9), with a 90% survival rate. All 41 children with failed portoenterostomy (and two without portoenterostomy) died or underwent liver transplantation at a median age of 0.8 years. In those where the portoenterostomy was felt to be successful, 80% are still alive and have not required liver transplantation. Survival without liver transplantation was noted to be significantly better at centers that had treated more than five cases yearly ($p = 0.005$). The conclusion drawn was that in the face of successful portoenterostomy, children would survive well into adolescence and beyond without the need for liver transplantation. The recommendation therefore was that children with biliary atresia should be treated in experienced centers to maximize the chance of successful surgery [2].

The second leading cause of liver disease requiring liver transplantation is metabolic liver disease in the under 2 years age group and acute hepatitis of unknown etiology leading to acute liver failure in the older age group (over 2 years) with the associated need for an urgent transplant within days of presentation (Figure 1B). The main indication for liver transplant in the teenage population is autoimmune liver disease that has not responded to treatment or cystic fibrosis-associated liver disease. Less common indications include malignancy, metabolic defects and, rarely, paracetamol overdose. This is in contrast to the adult population in which the leading indication for liver transplant is chronic hepatitis, primary biliary cirrhosis, hepatitis B, alcoholic liver disease and malignancy.

In inherited metabolic disease the indications for liver transplantation are variable depending on the underlying diagnosis. α1-antitrypsin for instance is a disorder with varying liver/lung involvement. Although 50–70% of children may develop progressive liver disease , only a third would require transplantation in childhood with the remainder surviving into adolescence/early adulthood without this need arising. Patients with tyrosinemia type 1 used to require liver transplantation following development of acute or chronic liver failure, and also for dysplastic change noted on biopsy following

abnormal scans. However following the introduction of Nitisone or 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexenedione – a compound with the ability to prevent the formation of toxic metabolites and resulting in a rapid clinical improvement – indications for transplant have changed and are now rarely needed in the absence of neoplastic change, which is still an indicator for transplant. Glycogen storage disease type I is not usually an indication for liver transplantation unless the medical management is not tolerated; there is development of multiple hepatic adenomata or poor quality of life. Glycogen storage diseases types III and IV may progress to cirrhosis, at which point liver transplantation is indicated due to hepatic dysfunction.

In older children with chronic liver disease, the main indication is failure of medical management of children with autoimmune liver disease or Wilson's disease. Children with autoimmune hepatitis type 2 are significantly more at risk of presenting with fulminant liver failure, which is often irreversible and will subsequently require liver transplantation.

Progression of liver transplantation: immunosuppression

Immunosuppression is needed to prevent graft rejection, but has significant side effects. There is no international standardized immunosuppression protocol, however most units use a combination of calcineurin inhibitor (CNI; e.g., tacrolimus or cyclosporin) with corticosteroids or mycophenolate mofetil (MMF). More recently, induction therapy with the addition of the IL-2 receptor antibody basiliximab has also been part of this regimen (the main purpose being protection during the fragile period when CNI levels are unstable, to prevent early rejection). Augmentation is usually with MMF or mTOR inhibitors, which allows for reduction of steroid and CNI use.

Changes over time: tacrolimus versus cyclosporin

Since its introduction in the early 1980s, cyclosporin transformed the way transplantation was managed. There was a marked reduction in episodes of rejection (compared with steroids alone) in the renal transplantation population, but it also facilitated other forms of transplantation including liver, heart and lung. Tacrolimus was introduced in the early/mid 1990s as a more effective therapy with less cosmetic side effects.

Two adult multicenter trials were performed in the mid 1990s to assess efficacy of tacrolimus in both Europe and the USA (European FK506 multicenter liver study group and US Multi-Center FK506 Liver Study Group) [3,4]. Both of these studies identified a reduction in the incidence of acute rejection; but with increased side effects such as renal impairment and development of diabetes mellitus.

In the pediatric population, ongoing trials evaluating the efficacy of tacrolimus versus cyclosporin-A support the change from cyclosporin to tacrolimus as there are fewer episodes of acute rejection and there is a better response to chronic rejection [1]. In 218 patients undergoing liver transplant between 1988 and 1996 tacrolimus improved patient and graft survival rates compared with cyclosporin with fewer episodes of acute rejection. However, there was an increased incidence of symptomatic Epstein–Barr infection (EBV) and possible post-transplant lymphoproliferative disease (PTLD) in patients treated with tacrolimus.

Kelly *et al.* presented data from a randomized controlled trial in 181 European children comparing efficacy of tacrolimus to cyclosporin microemulsion, which demonstrated that tacrolimus was more effective than cyclosporin in preventing steroid resistant rejection [5]. Median age was 22 months (range 9–56 months) in the tacrolimus group versus 17 months (range 9–54 months) in the cyclosporin-treated group. Patient and graft survivals were not significantly different at month 12 post-transplant. Rejection-free survival at the end of this study was 55.5% for patients on tacrolimus and 40.2% for patients on cyclosporin microemulsion ($p = 0.0288$). Event-free survival for patients at study end was significantly different at 94.0% for tacrolimus-treated versus 70.4% for cyclosporin-treated patients ($p < 0.0001$). Overall, incidence of adverse events did not differ between groups. This therefore supports the introduction of tacrolimus in preference to cyclosporin with regards to rejection and graft survival [5].

The introduction of basiliximab

Basiliximab is a chimeric mouse–human monoclonal antibody of the IgG1 isotype to the α chain (CD25) of the IL-2 receptor of T cells, acting as an antagonist at the IL-2 binding site of the p55 subunit IL-2 receptor (CD25) on activated T lymphocytes. Its use was first established to avoid the nephrotoxicity associated with the CNIs. Arora *et al.* reported a small case series of three patients with pre-existing renal impairment (mean cGFR pretransplant of 58.1 ml/min/m^2; calculated using modified Schwartz formula) who had basiliximab in combination with prednisolone and either cyclosporin or tacrolimus post-transplant, on the modified regime with basiliximab, the mean cGFR at 10 weeks had improved to 116 ml/min/m^2 [6]. Rejection occurred in two out of three patients; and one developed chronic rejection requiring retransplant. The authors concluded a larger study was necessary to fully evaluate the use of Basilixmab as its obvious benefits were evident [6].

In 2002, Neuhaus and colleagues did the first large-scale study of 381 adult patients undergoing liver transplant to further assess the efficacy of basiliximab.

The study showed that the proportion of transplant recipients with acute rejection episodes 6 or 12 months after transplantation was reduced by the addition of basiliximab to baseline, dual immunosuppression therapy with cyclosporin microemulsion and steroids. Comparison of survival curves at 6 months indicated statistical significance for the primary end points of first biopsy-confirmed rejection episode, death or graft loss ($p < 0.048$) [7]. This study, in common with previous studies of this drug, showed that basiliximab can be administered as a fixed two-dose intravenous bolus and is well tolerated, with no increase in adverse events or evidence of cytokine-release syndrome. Patients were administered cyclosporin from day 0 and demonstrated no evidence of renal dysfunction, suggesting that basiliximab complements dual immunosuppression therapy. Although increasing immunosuppression generally causes patients to be more susceptible to opportunistic infections, this study demonstrated no difference in the incidence of infection, including cytomegalovirus (a common post-transplant opportunistic infection), in the basiliximab and placebo groups, while serious fungal infections were reduced in the basiliximab-treated group. Post-transplantation malignancies, particularly lymphoproliferative disorders (e.g., PTLD), were similar between both treatment groups and no higher in the transplant population in general, which is reassuring and confirms the safety of the use of basiliximab in this population.

The introduction of everolimus: a revelation?

CNIs such as cyclosporin and tacrolimus remain the mainstay of organ transplant survival. However side effects remain a problem with these medications. Immunosuppressants of the mTOR inhibitor class (everolimus and sirolimus) act in synergy with CNIs. It is possible that use of these agents may reduce CNI side effects if used instead of CNIs or in combination with lower doses. Recently there has been a prospective, multicenter, open-label study in which new adult transplant recipients were randomized to three groups over a 2 year period:

- Everolimus initiation with tacrolimus elimination;
- Everolimus initiation with reduced-exposure tacrolimus;
- Standard tacrolimus control.

The results have been encouraging, however due to increased adverse effects seen in the tacrolimus elimination group (in particular incidence of acute rejection) – this arm of the study was terminated early. However, on comparison of the everolimus and tacrolimus group versus tacrolimus alone, it was found that the tacrolimus-alone group suffered increased renal impairment compared with the group that had tacrolimus and everolimus – with equal efficacy in both groups [8]. The authors concluded from this study that introduction of everolimus at day 30 post-transplant following induction with tacrolimus monotherapy achieved superior renal function when compared with tacrolimus alone, with a statistically significant difference between both groups at 1 year post-transplant, without any loss of efficacy.

How about steroid-free immunosuppression?

A number of pediatric centers have reported that steroids can be reduced after 6 months of transplantation [9]; however information regarding steroid-free immunosuppression from the outset is more variable and the subject of ongoing analysis [10]. Although corticosteroids have been part of immunosuppressive regimes since the early days of transplantation, steroid avoidance could be advantageous in pediatric recipients. Many authors have proposed that this could reduce susceptibility to infections, PTLD, dyslipidemia, hypertension, and growth failure. Steroid withdrawal (up to 3 months post-transplant) and steroid-free immunosuppression have been suggested as alternative regimes to avoid long-term steroid therapy in adult and pediatric liver transplantation. Gras *et al.* suggested combining tacrolimus with anti-IL-2R blockade (basiliximab) for steroid substitution [11], which they based on a recent European study of 181 pediatric liver transplant recipients by Kelly *et al.*, which had shown that use of tacrolimus leads to a lower incidence of acute rejection when compared with use of cyclosporin [5]. Gras *et al.* performed a study of 50 patients transplanted under steroid-free immunosuppression to assess efficacy of this regimen against standard protocols. This was based on a pilot study performed by the same center 2 years prior to this that compared liver-transplantation under steroid-free immunosuppression (tacrolimus and basiliximab only), with matched historical recipients (who received tacrolimus plus corticosteroids) as a historical control group. Rejection-free survival at 12 months was 75% in the tacrolimus–basiliximab group compared with 50% in the steroid group ($p = 0.05$). Growth in the 12 months following liver transplantation was also noted to be significantly better in the steroid-free group. They concluded that steroid avoidance was not harmful and combining tacrolimus with basiliximab as a steroid substitution was proposed as a safe alternative to tacrolimus and steroid immunosuppression [12]. Following assessment of metabolic, biochemical and histological parameters they concluded that steroid-free immunosuppression was a safe method of immunosuppression and beneficial at 3 years follow-up compared with children

undergoing transplantation under a classical protocol containing steroids.

Spada *et al*. also report the benefits of using a steroid-free immunosuppressive regimen [13]. They performed a randomized controlled trial directly comparing steroid and steroid-free immunosuppression and showed that the incidence of infection as well as acute rejection were higher in the steroid–tacrolimus group compared with the steroid-free group taking a combination of tacrolimus and basiliximab. Evans *et al*., however, demonstrated that following standard withdrawal of steroids at 3 months post-transplant, although live biochemistry was normal or near-normal, the incidence of graft fibrosis and presence of autoantibodies increased with time, therefore demonstrating the need for long-term surveillance [14].

How about cessation of immunosuppression?

Although still a controversial issue, there has been much interest in attempting to discontinue immunosuppressive agents altogether. This was first suspected as a possibility when patients who had been nonadherent with immunosuppressive agents or who had had them withdrawn for other reasons did not develop rejection, suggesting a process known as Operational Tolerance. This obviously poses an interesting area of debate in the pediatric population, who are, in contrast with adults, likely to live for many years post-transplant. They are also likely to reach reproductive age, and this therefore poses an attractive option for female patients of reproductive age, as the risk of immunosuppressive agents to the developing fetus together with unstable levels during pregnancy and also the ability to conceive are all factors to be considered in a patient who wishes to have a family

This proposal of cessation of immunosuppression has been suggested in response to studies in both patients who received cadaveric organs as well as living-related organs. Takatsuki *et al.* were able to demonstrate complete withdrawal of tacrolimus in nearly 40% of patients with a median drug-free period of 23.5 months (range: 3–69 months). A further 36.5% were being weaned at the time that the study was published. Importantly, they showed rejection only occurred in 25.4% of patients, of whom the rejection was successfully treated in all cases using standard therapy of intravenous steroids or reintroduction of CNIs [15]. Koshiba *et al.* likewise demonstrated that pediatric recipients of living-related organs were successfully weaned off of their immunosuppressive agents. Although the overall numbers were bigger, the actual percentage where complete cessation was achieved was less at 15%. They did suggest the mechanism behind operational tolerance as being similar to the fetomaternal mechanisms *in utero*, and suggested that this could be a possible mediator of operational tolerance, with a potential role of regulatory T-cells [16].

Most recently, a further pediatric study has shown that in 60% of pediatric patients from a multicenter trial including patients who had received a live-related graft – operational tolerance was achieved. Although the numbers are small, this is thought provoking and further studies are necessary to determine just how feasible this may be in the longer term and therefore discounting the potential issues surrounding conception as well as adherence – two issues central to the lives of young females [17].

Complications of liver transplantation: rejection

Acute rejection occurs by definition within the first 10 days post-transplant and has reduced since the introduction of tacrolimus. The use of IL-2-receptor blocking antibodies such as basiliximab/daclizumab has also transformed the event-free survival of children following liver transplant, demonstrating improved graft function and a reduction in the risk of rejection in the immediate post-transplant period [5,13,18].

Late onset acute rejection is defined as occurring up to 5 years after liver transplant. Recent findings in SPLIT study found approximately one in five patients who avoided early acute rejection developed late-onset acute rejection between 1 and 5 years [19].

Chronic rejection is a cause of long-term graft dysfunction and cirrhosis with eventual graft loss. The SPLIT registry focussed on graft loss in children followed more than 1 year post-transplantation and they found 37% lost grafts because of chronic rejection and 11% due to acute rejection. Using BANFF criteria for graft rejection they used a definition of chronic rejection as "minimal histological changes affecting the majority of bile ducts with or without duct loss, foam cell obliterative arteriopathy or bile duct loss affecting >50% of portal tracts" [19–23]. The SPLIT group also found that steroid resistance was a poor prognostic sign and associated with late graft loss, as was having more than one episode of rejection. Inevitably adherence plays a role in this, with children who are nonadherent having increased risk of rejection episodes and long-term complications.

Other complications & long term side effects of immunosuppression

Post-transplant diabetes mellitus is an important complication amongst patients receiving immunosuppression. It also, as a result, can have a long-term impact on graft function. A recent interim adult study from Taiwan looking at complications of immunosuppression in the post-transplant population of 101 patients (liver recipients n = 13, renal n = 77 and cardiac n = 11) receiving

the newer CNIs (tacrolimus) and monoclonal antibodies (basiliximab) and a more traditional combination of MMF, cyclosporin-A and corticosteroids [24]. The use of CNIs has been implicated in an increased risk of developing new-onset diabetes mellitus (NODM) following its introduction. Although this study focused primarily on transplant recipients who took cyclosporin in combination with other medications as their immunosuppressant regime, it is unlikely that it is the cyclosporin that is responsible for development of diabetes mellitus. A further study by Lorho *et al*. has suggested that tacrolimus is also responsible for the development of diabetes mellitus but that this can be reversed on discontinuation of tacrolimus and introduction of cyclosporin-A [25]. They recently performed a 12-month pilot study of 39 liver recipients with NODM who were converted from tacrolimus to cyclosporin. At the end of the 12 month study, the blood sugars and therefore incidence of NODM had resolved to normal levels in 36% of patients. The use of corticosteroids is not solely responsible for the incidence of development of NODM either, as the same study stopped steroids in nine patients with NODM; however, only three of them showed any improvement in blood glucose levels. In pediatrics the prevalence of post-transplant diabetes is around half that seen in the adult population, the reasons for which are unclear [26,27]. Risk factors for developing post-transplant diabetes include racial background (those of Afro-Caribbean decent are high risk), primary diagnosis of cystic fibrosis or primary sclerosing cholangitis and acute hepatic necrosis.

Cardiovascular disease is also a risk post-transplant as it is known that immunosuppression can lead to obesity, hypertension and dyslipidemia. The SPLIT data showed that obesity (defined as a weight >95th percentile) was present in 12% of liver transplant patients included in their study, with hypercholesterolemia noted in 7% [20].

Renal dysfunction

CNIs have improved survival after liver transplantation; however, they can also have a detrimental effect on renal function. Arora-Gupta *et al*. did a retrospective assessment of the long-term renal function in children receiving CNIs over a 10-year period [28]. Calculated glomerular filtration rate (cGFR) was calculated pre-transplant as well as at 3, 6 and 12 months post-transplant and annually thereafter. 113 patients (65 males, 48 females) were followed up. They noted that there was a significant deterioration in renal function at 3 months compared with the pretransplant values ($p = 0.001$). By 12 months following the reduction in immunosuppression dosage, renal function had stabilized, and in fact had shown a slight improvement reaching 76% of the pretransplant value at 5 years ($p < 0.001$). Children who were <1 year of age at the time of transplant had a significantly better recovery in their renal function than older children ($p = 0.02$). No association was seen with gender, the type of immunosuppression or the underlying diagnosis. Despite an initial reduction in cGFR, which was felt to be associated with the period of maximum immunosuppression, long-term low dose CNI therapy was not associated with ongoing deterioration of renal function, and significantly also not in children who were transplanted at a young age.

More recently Basso *et al*. have assessed the use of sirolimus as a rescue formulation for children experiencing acute and chronic rejection as well as those with a nephropathy caused by immunosuppressant use [29]. Indications for the use of sirolimus were histologically demonstrated, standard treatment-resistant rejection, early chronic rejection and immunosupressant-induced nephropathy with an associated intolerance to MMF. It was found that as a result, aspartate aminotransferase normalized in ten out of 12 patients with acute rejection. In those with chronic rejection, aspartate aminotransferase normalized in 50%. Those with renal impairment showed improvement in their creatinine levels ($p = 0.03$). From this, they concluded that sirolimus was an effective rescue therapy for patients with rejection (both acute and chronic) as well as those with renal impairment as a result of traditional regimes.

Infections & the post-transplant population

Cytomegalovirus (CMV) is a risk in the post-transplant period at any stage and infection rate may be as high as 40% with mortality as high as 19% [30]. Patients are at highest risk from transmission from the donor organ in an infection-naive patient. These recipients need careful management peritransplant whilst serology is pending and consideration given to extending the course of antiviral treatment in those at risk of this complication. CMV produces a varied disease phenotype from asymptomatic derangement of liver function tests up to severe respiratory illness and graft dysfunction. In the pretransplant period it is important to ascertain the recipients carrier state as reactivation of latent infection is also a possibility [31].

EBV is a serious risk in immunosuppressed patients regardless of regime. The correlation between development of infection, EBV-mediated PTLD and type of immunosuppressant has remained a topic of discussion. Although initial reports suggested that PTLD was more common with tacrolimus, it is more likely to be correlated with dose and exposure than individual drugs [32–34]. EBV infection and PTLD are more common after primary EBV infection in children who are seronegative at transplant, which includes most of the pediatric transplant population. Most children are infected in the first year post-transplant and present with a spectrum

of symptoms from mild-nonspecific viral illness up to PTLD. Organ dysfunction (liver, lung, gastrointestinal) and hematological manifestation (thrombocytopenia, leukopenia, hemolytic anemia) may also occur [35].

Malignancy

Patients post liver transplantation may be at risk of *de novo*, donor-transmitted or recurrent malignancies. Recurrent and *de novo* malignancies have been found to be second (only to cardiovascular complications) as a leading cause of late death in transplant recipients. The increased incidence rate of *de novo* malignancies may be due to immunosuppression. Chronic viral infections with Epstein–Barr virus are associated with post-transplant lymphoproliferative disease, skin cancers (including squamous cell carcinoma and Kaposi's sarcoma). Increased incidence of *de novo* malignancy requires careful long-term screening protocols.

Under the surface: long-term histological changes in pediatric transplants

As survival post-transplant improves, the challenge now remains as to how to assess the late post-transplant surveillance biopsies. The severity of changes is often dependent on the original indication for transplant. This is less important for pediatric transplant recipients as few pediatric diseases are likely to recur (e.g., biliary atresia). It was first noted, that progressive hepatitis and fibrotic changes were first noted by Evans *et al.* in 2006 when assessing surveillance liver biopsies following liver transplantation [18]. They reported an increasing incidence of indeterminate graft hepatitis, comprising histologically demonstrated chronic hepatitis in association with autoantibody production, which was found to be a common feature in the pediatric population studied. In the review, 158 asymptomatic children underwent protocol liver biopsies (113, 135, and 64 at 1, 5 and 10 years post liver transplant, respectively). All the findings were correlated. All the patients received cyclosporin A as primary immunosuppression in addition to corticosteroids, which were weaned by 3 months as standard post-transplant. Normal or near-normal histology was reported in 68% at 1 year, 45% at 5 years, and 31% at 10 years, the commonest abnormality found being chronic hepatitis. The incidence of this increased with time, going from being found in 22% of samples at 1 year up to 64% of samples by 10 years, which was statistically significant. The incidence of fibrosis also increased at the same time from 52% at 1 year to 91% by 10 years post-transplant, and 15% had progressed to cirrhosis by 10 years post-transplant. Hubscher *et al.* noted that 'normal' changes seen in pediatric samples – such as mild fibrosis or inflammatory changes are frequently seen in surveillance biopsies in children with normal or near-normal liver biochemistry [36]. Hubscher *et al.* noted that abnormal histological findings were found in between 69–97% of all biopsies in late post-transplant biopsies following review of a number of similar studies. He also notes that these changes might be due to late rejection due to suboptimal immunosuppression or noncompliance associated with adolescence [37]. Due to the risk of under-immunosupression, long-term surveillance is vital, especially in steroid-free regimes. Late rejection changes seen on biopsies include a predominantly mononuclear portal inflammatory infiltrate, prominent interface hepatitis and prominent lobular hepatitis. These features are similar to those seen in autoimmune hepatitis and chronic viral hepatitis [38,39]. The Banff Working Group likewise emphasized the need for surveillance biopsies both prior to, and after weaning of immunosuprssive agents in those patients that had their immunosuppression ceased (i.e., those described as having operational tolerance). This protocol was developed in response to previous reports regarding the insensitivity of serum biochemistry in detecting the histological changes previously described. They also suggested using non-invasive biomarkers (such as autoantibodies, gammaglobulins) that could be used to identify those patients who would tolerate withdrawal of immunosuppression [40].

Vascular and biliary complications are frequent in pediatric transplant recipients, which are related to the size of vessels and ducts [41]. Histological features include fibrous portal expansion, progressive periportal fibrosis and possible biliary cirrhosis. Treatment options include interventional radiological procedures including percutaneous transhepatic cholangiography (with option to place a biliary drainage catheter) or retransplantation.

Rehabilitation post-transplant

Many children who undergo a liver transplant have a background of chronic liver disease and malnutrition. Children with cirrhosis will have fat soluble vitamin deficiencies secondary to fat malabsorption, abnormal nitrogen metabolism and increased energy requirements. Many studies have shown that nutritional rehabilitation takes place within 1 year post-transplant with catch-up growth. Growth may be impaired by steroid therapy (by up to 2 years) and some never reach their full growth potential. Prolonged steroid exposure and underlying metabolic disease (α1 anti-trypsin deficiency, urea cycle defects) may lead to linear growth impairment in <10th percentile [42].

Endocrine complications may also be apparent. End-stage liver disease leads to growth failure, pubertal delay and hepatic osteodystrophy [43,44]. As a result, post-transplant, it is advisable that vitamin and nutritional mineral levels are monitored as recovery can be slow and children are at risk of fractures, scoliosis and growth

failure. Vitamin supplementation should be considered, especially in those with previous cholestatic liver disease (due to prolonged malabsorption) and in those with radiological evidence of metabolic bone disease, as adequate nutrition and additional supplementation is required, with nasogastric feeds if necessary.

Psychosocial adjustment post-transplant

The recent SPLIT registry international survey of transplant recipients demonstrated that a third of transplant recipients missed 10 days or more from education in the previous year and nearly a fifth (18%) missed more than 20 days. They noted absence was more likely in the older children and especially in those with recent transplant [45]. It was felt by the study that some of the school absence was due to hospital follow up clinic appointments prompting their recommendation of arranging follow-up visits at times that would not interfere with education. It was also reported that in the older children, 16% reported symptoms that would be consistent with a diagnosis of post-traumatic stress disorder [46]. Similar symptoms were also reported in parents/carers [43,47]. Children also have expressed anxiety regarding medications, citing changes to physical appearance and parental conflict as reasons for possible nonadherence [48]. So severe is the post-transplant anxiety that when questioned relating to quality of life – many described their quality of life as being as poor as a child with life-threatening malignancy.

Neurocognitive development

Post-transplantation, a number of children continue to have a degree of neurocognitive impairment, long after physical impairments have resolved [49]. The etiology is unknown, but may be related to poor nutritional state, and/or poor school attendance prior to transplantation.

Adolescent transition

Nonadherence to immunosuppression leads to graft dysfunction/failure and death. This is a particular risk in the adolescent population. Nonadherence has been significantly associated with retransplantation and death secondary to chronic rejection in a number of studies. One such study by Berquist *et al.* described groups within this population and risk factors associated with nonadherence such as those teenagers from lower socioeconomic class ($p < 0.025$), older age at transplant ($p < 0.005$) and episodes of late acute rejection ($p < 0.001$). They proposed that a targeted intervention for high-risk groups was a realistic goal [50]. Perhaps most worrying was their prevalence rate of 38.2% of nonadherence found in their study. In order to address this, much work has gone into education and assessment pre-transplant, with involvement of the multidisciplinary teams (comprising social support team, education, psychology and specialist nursing teams) in order to ascertain level of understanding and identify those patients at particular risk of developing risky behaviors such as nonadherence to medications.

Lurie *et al.* made an assessment of the risk factors for nonadherence in the pediatric population [51]. They report three cases of fatal nonadherence. They identified risk factors for nonadherence including: substance abuse, child abuse (physical and sexual), single parent family situation, psychiatric disorder and history of education failure (leaving school prematurely without completing education).

This has led to a greater emphasis of 'transitioning' patients to adult services that are not based on chronological age. Instead there is a gradual preparation program from 12 years in which there are specific teenage/adolescent clinics joint with adult and pediatric services, which supports the young person with a gradual change in emphasis from being the child with liver disease to being a young adult who is independent and takes control of their disease.

Annunziato *et al.* looked at the adherence and medical outcomes of pediatric liver transplant recipients after undergoing transition into adult services [52]. They reviewed 14 patients pre- and post-transition assessing medical outcomes, and compared them with patients solely in pediatric or adult care. They assessed medication adherence as standard deviation of tacrolimus blood level. They found that tacrolimus adherence was significantly decreased in the patients post-transition. They also found that adherence in the post-transition group was also worse than the sole adult or sole pediatric groups. Their conclusion was that the transition period was a period of vulnerability with regards to 'getting it right' and they have recommended a larger study. In 2010, Fredericks *et al.* looked at the assessment of 'readiness' for transition [53]. They concluded based on a number of parameters including perceived and measured self-management skills, regimen knowledge, psychosocial adjustment and adherence, that there was a positive correlation between them all except demonstrated skills and nonadherence with age. There was a positive correlation between trough medication levels and self-management skills as well as with increased responsibility for self-management medication tasks. Nonadherence is associated with having an increased risk of developing medical complications but is felt to be potentially modifiable. They therefore concluded that interventions that promote self-management skills and adherence should be encouraged and form an essential part of the transition planning process.

Recent guidance from the International Society of Nephrology and the International Pediatric Nephrology Association can be applied to the pediatric liver transition service. They have suggested a number of concepts including gradually introducing the idea of transitioning, identifying transition champions to co-ordinate

the process, individualized transition plans, inclusion of family members in the process, peer support services and social networking – all have been shown to be of benefit to the transition process [54]. The overwhelming message from all the recent literature would suggest the key to a successful transition is to engage with the young person and give them a sense of self management. They need to take responsibility for their own health care and it is the physician's duty to guide them to do this. There needs to be a partnership between adult and pediatric services in order to facilitate this process.

As operative techniques are modified and optimized we move from evaluating short-term goals such as 1- and 5-year survival, but move to longer term goals such as 10–15 years and beyond. Our emphasis is now on how best to maintain the graft longer, focusing on preservation of organ function and prevention of unacceptable side effects. We need to modify immunosuppressants to reduce other organ dysfunction, reduce cardiovascular risk and risk of malignancy and we need to prepare our patients for adult life.

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Executive summary

Liver disease in the paediatric population

- The leading cause of liver disease in early childhood is cholestatic liver disease. In older children metabolic and autoimmune diseases predominate as a cause.

Progression of liver transplantation

- To prevent graft dysfunction, immunosuppression is needed. There is no standardised protocol, however current combinations widely used worldwide include a calcineurin inhibitor (cyclosporin A/tacrolimus) with corticosteroid or mycophenolate mofetil.
- More recently IL-2 receptor antibodies have been used and have become a critical adjunct to prevent early rejection.

Complications of liver transplantation

- There are a number of recognised complication including rejection, new onset diabetes mellitus, cardiovascular disease, renal dysfunction and infection (particularly Cytomegalovirus and Epstein–Barr virus) remain a serious risk in immunosuppressed patients.
- Other complications include: malignancy – *de novo*, donor-transmitted or recurrent and sometimes associated with viral infection – and indeterminate graft hepatitis, which has been demonstrated in long-term transplant survivors (10 years +) and is found in routine surveillance biopsy on the background of normal or near-normal serum biochemistry.

Rehabilitation post-transplant

- Nutritional rehabilitation takes place over the first year post-transplant. Long-term growth may be impaired by corticosteroid use, which is another reason for trying to reduce this agent's use in the paediatric population.

Psychosocial adjustment

- Psychological stress may also lead to nonadherence with medications.

Adolescent transition

- Much work has been put in recently to improve the transition process for young people into adult care; for example, transition nurses, dedicated multidisciplinary teams as well as identifying those particularly at risk so that services can be targeted to these individuals.

References

Papers of special note have been highlighted as:

- ▪ of interest

1 Cao S, Cox KL, Berquist W *et al.* Long-term outcomes in pediatric liver recipients: comparison between cyclosporin A and tacrolimus. *Pediatr. Transplant.* 3(1), 22–26 (1999).

2 McKiernan PJ, Baker AJ, Lloyd C *et al.* British Paediatric Surveillance Unit study of biliary atresia: outcome at 13 years. *J. Ped. Gastrol. Nutr.* 48, 78–81 (2009).

3 No authors listed. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. *Lancet* 344, 423–428 (1994).

4 No authors listed. A comparison of tacrolimus (FK506) and cyclosporin for immunosuppression in liver transplantation. The US Multicenter FK506 Liver Study Group. *N. Engl. J. Med.* 331, 1110–1115 (1994).

5 Kelly D, Jara P, Rodeck B *et al.* Tacrolimus and steroids versus cyclosporin microemulsion, steroids, and azathioprine in children undergoing liver transplantation: randomised European multicentre trial. *Lancet* 364(9439), 1054–1061 (2004).

▪ **One of the first large-scale multicentre studies making a direct comparison of tacrolimus and cyclosporin.**

6 Arora N, McKiernan PJ, Beath SV *et al.* Concomitant basiliximab with low-dose calcineurin inhibitors in children post-liver transplantation. *Pediatr. Transplant.* 6, 214–218 (2002).

▪ **Heralds the use of monoclonal antibodies in the liver-transplant population.**

7 Neuhaus P, Clavien P, Kittur D *et al.* Improved treatment response with basiliximab immunoprophylaxis after liver transplantation: results from a double-blind randomized placebo-controlled trial. *Liver Transpl.* 8, 132–142 (2002).

8 De Simone P, Nevens F, De Carlis L *et al.* Everolimus with reduced tacrolimus improves renal function in *de novo* liver transplant recipients: a randomized controlled trial. *Am. J. Transplant.* 12, 3008–3020 (2012).

9 Alabraba E, Nightingale P, Gunson B *et al.* A re-evaluation of the risk factors for the recurrence of primary sclerosing cholangitis in liver allografts. *Liver Tranpl.* 15, 330–340 (2009).

10 Lerut J, Bonaccorsi-Riani E, Finet P *et al.* Minimization of steroids in liver transplantation. *Transpl. Int.* 22, 2–19 (2009).

11 Gras J, Gerkens S, Beguin C *et al.* Steroid-free, tacrolimus-basiliximab immunosuppression in pediatric liver transplantation: clinical and pharmacoeconomic study in 50 children. *Liver Transpl.* 14, 469–477 (2008).

■ **Examines the potential for steroid-free immunosuppression.**

12 Redding R, Gras J, Sokal E *et al.* Steroid-free liver transplantation in children. *Lancet* 362(9401), 2068–2070 (2003).

13 Spada M, Petz W, Bertani A *et al.* Randomized trial of basiliximab induction versus steroid therapy in pediatric liver allograft recipients under tacrolimus immunosuppression. *Am J Transplant.* 6, 1913–1921 (2006).

14 Evans HM, Kelly DA, McKiernan PJ *et al.* Progressive histological damage in liver allografts following pediatric liver transplantation. *Hepatology* 43, 1109–1117 (2006).

■ **One of the first studies to describe the abnormal histology findings in routine surveillance biopsies in young people post-transplant, especially in the face of normal biochemical markers.**

15 Takatsuki M, Uemoto S, Inomata Y *et al.* Weaning of immunosuppression in living donor liver transplant recipients. *Transplantation* 72(3), 449–454 (2001).

16 Koshiba T, Li Y, Takemura M *et al.* Clinical, immunological, and pathological aspects of operational tolerance after pediatric living-donor liver transplantation. *Transplant. Immunol.* 17(2), 94–97 (2007).

■ **Introduces the concept of operational tolerance.**

17 Feng S, Ekong UD, Lobritto SJ *et al.* Complete immunosuppression withdrawal and subsequent allograft function among pediatric recipients of parental living donor liver transplants. *JAMA* 307(3), 283–293 (2012).

18 Kelly D. Safety and efficacy of tacrolimus in pediatric liver recipients. *Paediatr. Transplant.* 15, 19–24 (2011).

19 Ng V, Fecteau A, Shepherd R *et al.* Outcomes of 5-Year survivors of pediatric livertransplantation: report on 461 children from a North American multicenter registry. *Paediatrics* 122(6), 1128–1135 (2008).

20 Kelly DA. Current issues in pediatric transplantation. *Pediatr. Transplant.* 10, 712–720 (2006).

21 Wallot M, Mathot M, Janssen M *et al.* Long-term survival and late graft loss in pediatric liver transplant recipients – a 15 year single-center experience. *Liver Transpl.* 8(7), 615–622 (2002).

22 Aw M, Taylor R, Verma A. Basiliximab (Simulect) for the treatment of steroid-resistant rejection in pediatric liver transpland recipients: a preliminary experience. *Transpl.* 75, 796–799 (2003).

23 Soltys KA, Mazariegos GV, Squires RH *et al.* Late graft loss or death in pediatric livertransplantation: an analysis of the SPLIT database. *Am. J. Transpl.* 7, 2165–2171 (2007).

24 Tian YF, Tsai CS, Lee PC *et al.* New-onset diabetes mellitus in cyclosporine-treated organ transplant patients in Taiwan: interim analysis (6 months) of postmarketing surveillance. *Transplant Proc.* 44(3), 661–666 (2012).

25 Lorho R, Hardwigsen J, Dumortier J *et al.* Regression of new-onset diabetes mellitus after conversion from tacrolimus to cyclosporine in liver transplant patients: results of a pilot study. *Clin. Res. Hepatol. Gastroenterol.* 35(6–7), 482–488 (2011).

26 Marchetti P. New-onset diabetes after liver transplantation: from pathogenesis to management. *Liver Transpl.* 11, 612–620 (2005).

27 Pageaux G, Faure S, Bouyabrine H *et al.* Long term outcomes of liver transplantation: diabetes mellitus. *Liver Transpl.* 15(Suppl. 2), S79–S82 (2009).

28 Arora-Gupta N, Davies P, McKiernan P *et al.* The effect of long-term calcineurin inhibitor therapy on renal function in children after liver transplantation. *Pediatr. Transplant.* 8, 145–150 (2004).

■ **Offers understanding on the effect of calcineurin inhibitors on renal function.**

29 Basso M-S, Subramaniam P, Tredger M *et al.* Sirolimus as renal and immunological rescue agent in pediatric liver transplant recipients. *Pediatr. Transplant.* 15, 722–727 (2011).

30 Michaels MG, Fonseca-Aten M, Green M *et al.* Respiratory syncytial virus prophylaxis: a survey of pediatric solid organ transplant centers. *Pediatr. Transplant.* 13, 451–456 (2009).

31 Penkowera L, Dewb A, Ellisc D *et al.* Psychological distress and adherence to the medical regimen among adolescent renal transplant recipients. *Am. J. Transplant.* 3, 1418–1425 (2003).

32 Reyes J, Tzakis A, Green M *et al.* Post-transplant lymphoproliferative disorders occurring under primary FK 506 immunosuppression. *Trans. Proc.* 23, 3044–3046 (1991).

33 Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R *et al.* An increased incidence of Epstein–Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. *Transplantation* 59, 524–529 (1995).

34 Reding R, Wallemacq P, Lamy P *et al.* Conversion from cyclosporine to Fk506 for salvage of immunocompromised pediatric liver allografts efficacy, toxicity, and dose regimen in 23 children. *Transplantation* 57(1), 93–100 (1994).

35 Preiksaitis JK. New developments in the diagnosis and management of post-transplant lymphoproliferative disorders (PTLD) in solid organ transplant recipients. *Clin. Infect. Dis.* 39, 1016–1023 (2004).

36 Hubscher S. What does the long term liver allograft look like for the pediatric recipient? *Liver Transpl.* 15(2), S19–S24 (2009).

■ **Assesses the long-term histological features of paediatric transplanted livers.**

37 Venkat VL, Nick TG, Wang Y, Bucuvalas JC. An objective measure to identify pediatric liver transplant recipients at risk for late allograft rejection related to non-adherence. *Pediatr Transplant.* 12, 67–72 (2008).

38 Banff Working Group; Demetris AJ, Adeyi O, Bellamy CO *et al.* Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology* 44, 489–401 (2006).

39 Sundaram S, Melin-Aldana H, Neighbors K *et al.* Histologic characteristics of late cellular rejection, significance of centrilobular injury,and long-term outcome in pediatric liver transplant recipients. *Liver Transpl.* 12, 58–64 (2006).

40 Banff Working Group on Liver Allograft Pathology. Importance of liver biopsy findings in immunosuppression management: biopsy monitoring and working criteria for patients with operational tolerance. *Liver Transpl.* 18, 1154–1170 (2012).

■ **Introduces the concept of cessation of immunosuppression.**

41 Jain A, Costa G, Marsh W *et al.* Thrombotic and nonthrombotic hepatic artery complications in adults and children following primary liver transplantation with long-term follow-up in 1000 consecutive patients. *Transpl. Int.* 19, 27–37 (2006).

42 Alonso E, Shepherd R, Martz K *et al.* Linear growth patterns in prepubertal children following liver transplantation. *Am. J. Transpl.* 9, 1389–1397 (2009).

43 Alonso EM. Growth and development considerations in pediatric liver transplant. *Liver Transpl.* 14, 585–591 (2008).

44 Hogler W, Baumann U, Kelly D. Growth and bone health in chronic liver disease and following liver transplantation in children. *Pediatr. Endocrinol. Rev.* 7(3), 266–274 (2010).

45 Gilmour S, Adkins R, Liddell G *et al.* Assessment of psychoeducational outcomes after pediatric liver transplant . *Am. J. Transpl.* 9, 94–300 (2009).

46 Mintzer LL, Stuber ML, Seacord D *et al.* Traumatic stress symptoms in adolescent organ transplant recipients. *Pediatrics* 115, 1640–1644 (2005).

47 Walker AM, Harris G, Baker A *et al.* PTSD responses following liver transplantation in older children. *J. Child Psychol. Psychiatry* 40, 363–374 (1999).

48 Fredericks EM, Lopez MJ, Magee JC *et al.* Psychological functioning, non-adherence and health outcomes after pediatric liver transplantation. *Am. J. Transplant*. 7(8), 1974–1983 (2007).

49 Sorensen L, Neighbors K, Martz K *et al.* Cognitive and academic outcomes after pediatric liver transplantation: functional outcomes group (FOG) results. *Am. J. Transpl.* 11, 303–311 (2011).

50 Berquist RK, Berquist WE, Esquivel CO *et al.* Adolescent non-adherence: prevalence and consequences in liver transplant recipients. *Pediatr. Transpl.* 10(3), 304–310 (2006).

51 Lurie S, Shemesh LS, Sheiner PA *et al.* Non-adherence in pediatric liver transplant recipients – An assessment of risk factors and natural history. *Pediatr Transpl.* 4, 200–206 (2000).

52 Annunziato RA, Emre S, Shneider B *et al.* Adherence and medical outcomes in pediatric liver transplant recipients who transition to adult services. *Pediatr Transpl.* 11(6), 608–614 (2007).

■ **Offers understanding on the transition services available to pediatric liver transplant recipients, and its impact on adherence.**

53 Fredericks EM, Dore-Stites D, Well A *et al.* Assessment of transition readiness skills and adherence in pediatric liver transplant recipients. *Pediatr Transpl.* 14, 944–953, (2010).

54 Watson AR, Harden PN, Ferris M,*et* al. Transition from pediatric to adult renal services: a consensus statement by the International Society of Nephrology (ISN) and the International Pediatric Nephrology Association (IPNA). *Kidney Int.* 80, 704–707 (2011).