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Meeting Report

Improving Long-Term Outcomes After Liver Transplantation in Children

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The objective was to review the current state of knowledge and recommend future research directions related to long-term outcomes for pediatric liver transplant recipients. A 1-day Clinical Research Workshop on Improving Long-Term Outcomes for Pediatric Liver Transplant Recipients was held on February 12, 2007, in Washington, DC. The speaker topics were germane to research priorities delineated in the chapters on Pediatric Liver Diseases and on Liver Transplantation in the *Trans-NIH Action Plan for Liver Disease Research.* Issues that compromise long-term well-being and survival but are amenable to existing and new research efforts were presented and discussed. Areas of research that further enhanced the research priorities in the *Action Plan for Liver Disease Research* included collection of longitudinal data to define emerging trends of clinical challenges; identification of risk factors associated with long-term immunosuppression complications; development of tolerance-inducing regimens; definition of biomarkers that reflect the level of clinical immunosuppression; development of instruments for the measurement of health wellness; identification of risk factors that impede growth and intellectual development before and after liver transplantation and identification of barriers and facilitators that impact nonadherence and transition of care for adolescents.

Key words: Children, liver transplantation, outcomes

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Despite favorable long-term survival, patients, their families and providers face major challenges after liver transplantation (LT), particularly related to the life-long immunosuppression and follow-up currently necessary posttransplant. The Liver Diseases Research Branch of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the Office of Rare Diseases (ORD) organized and sponsored a meeting on improving long-term outcomes after LT in children held on February 12, 2007, in Washington, DC. The conference topics were relevant to research priorities delineated in the chapters on pediatric liver diseases and on liver transplantation in the *Trans-NIH Action Plan for Liver Disease Research* (http://liverplan.niddk.nih.gov). This summary serves as a compilation of the presentations and discussions that transpired during the conference.

Session 1: Current Issues Confronting Long-Term Survival After Pediatric Liver Transplantation

Patient and graft survival

Presenters: Dr. Sue McDiarmid (University of California, Los Angeles), Dr. John Magee (University of Michigan) and Dr. George Mazariegos (University of Pittsburgh)

Based on data retrieved from the Scientific Registry of Transplant Recipients (SRTR), in 2006, there were 5500 patients still living who had received a LT as a child. While the children younger than 6 years at transplant had the highest rate of early graft loss, evaluation of survival curves revealed that over time, graft loss for the youngest children reached a plateau. In contrast, older children had a lower rate of early graft loss, yet their survival curves for graft loss after 1 year had a steeper slope, indicating the risk of graft loss in the subsequent years exceeded that of the younger children.

Children with noncholestatic cirrhosis have equal risk in the short term but higher risk of long-term graft failure compared to those with cholestatic cirrhosis. Children with malignancy had significantly higher risks of early and late graft loss, while the impact of acute liver failure was restricted to short-term outcomes, likely reflecting increased CNS-related complications. In the short-term model, younger age was associated with a greater risk of graft loss. Status at transplant, graft source and type of graft predicted

short-term but not long-term graft loss. Era of transplant was also associated with short-term graft survival, with the risk decreasing over more recent years. Factors that were predictive of late graft loss included older age at transplant, race and type of insurance, suggesting a role for development or socioeconomic factors. Interestingly, there was no impact of era on long-term risk suggesting that the progress made in improving short-term results has not carried over to factors responsible for long-term outcomes. The interactions among age at transplant, age at graft loss and time since transplant were considered to evaluate impact of age and time on the risk of graft loss. After adjusting for donor age and year of transplant, the recipient's current age was more predictive of graft loss than the age at transplant. These findings suggest a strong role of age in determining long-term outcomes. Whether increased risk for late graft loss reflected differences in immunobiology with age or nonadherence remains to be determined.

Outcomes based on 872 patients from the SPLIT registry who survived the first year posttransplant were reviewed. Five-year graft and patient survival were 89.2% and 94.2%, respectively (1). Acute (11%) and chronic (37%) rejection accounted for nearly half the 35 late graft losses that led to retransplantation. Other causes of graft loss included late complications related to hepatic artery thrombosis (11%) and biliary complications (9%). Primary diagnosis of malignancy or fulminant hepatic failure, reoperation in the first 30 days following initial transplantation, more than five hospital admissions over the course of the first posttransplant year and steroid-resistant rejection were independent factors associated with an increased risk of late graft loss. Among the children who survived the first year, there were 34 deaths. Recurrent malignancy most commonly due to hepatoblastoma accounted for six deaths (18%) and was the leading cause of late death. Sepsis (15%), multisystem organ failure (15%) and posttransplant lymphoproliferative disorders (PTLD) (15%) were the other causes of late mortality (1). Primary diagnosis of malignancy or acute liver failure, a weight deficit at transplant, history of hepatic artery thrombosis and more than five hospital admissions in the first year following transplant were independent factors associated with late death. While allograft loss occurred as a result of inadequate immunosuppression, late posttransplant mortality was linked to complications of excess immunosuppression. This analysis highlighted a clear opportunity to develop predictive assays or biomarkers that may help optimize and individualize immunosuppression in children. The lack of alignment of predictors for late graft loss between the SPLIT and SRTR registries is of uncertain origin but may reflect differences in the size of the data set, operational definitions or data elements. Even so, the association of late graft loss with inadequate immunosuppression noted in the analyses of the SPLIT dataset and the increased risk based on the recipient's current age note in the analyses from the SRTR dataset support a role for nonadherence in late graft loss.

Chronic allograft dysfunction

Presenters: Dr. Ross Shepherd (St Louis Children's Hospital)

Many factors contribute to late liver allograft dysfunction including biliary and vascular complications, rejection, infection, drug-associated hepatotoxicity and recurrent disease but perhaps the most intriguing is the chronic hepatitis. Evans et al. recently published the results of protocol liver biopsies performed in 158 patients 5 or more years after LT treated with cyclosporine as primary immunosuppression (2). Chronic hepatitis was defined by a predominantly portal-based mononuclear inflammation while bile duct lesions or vascular changes characteristic of acute or chronic rejection were minimal or absent. Chronic hepatitis was present in 43% of protocol biopsies at 5 years and in 64% of biopsies at 10 years posttransplant. Serum aminotransferase levels were not different from patients with chronic hepatitis compared to normal liver biopsies, but autoantibodies were present in 10% of children with normal liver biopsies compared to 80% of patients with chronic hepatitis, raising concern of immune-mediated graft injury. The incidence of fibrosis increased with time suggesting a progressive injury. In a similar study of long-term survivors, Fouquet et al. reported fibrosis and/or chronic rejection in 73% of liver biopsies from patients 10 years after transplantation for biliary atresia (3). It remains to be determined if these findings reflect a form of late allograft rejection or a distinct *de novo* autoimmune hepatitis.

Session 2: Immunobiology and Immunosuppression in Pediatric Liver Transplantation

Immune monitoring and biomarkers

Presenter: Dr. Rakesh Sindhi (University of Pittsburgh)

While the link between drug level and efficacy/toxicity is defined at a population level, individual risk is difficult to assess. Dr Sindhi described a mixed lymphocyte reaction-based assay as a tool to predict the risk of allograft rejection. Overexpression of CD154, a marker for IFN*+ antigen-specific T-cell function, and underexpression of CTLA4, a marker of T3 regulatory function, marked the rejection-prone recipient, while overexpression of CTLA4 and underexpression of CD154 characterized the rejection-free recipient (4,5). They defined the 'immunoreactivity index' as the fraction of donor-induced CD154+T cells to those induced by third-party antigens. If CD154 expression was increased in donor-induced cells as compared to cells induced by third-party antigens, the immunoreactivity index was >1, indicating an increased risk of rejection. If the suppressive T-cell marker CTLA4 was increased in donor-induced versus third-party antigen-induced cells, then the risk of rejection was decreased. In 49 pediatric transplant recipients, the assays predicted rejector/nonrejector status with sensitivity and specificity >93%. While these assays

may measure the risk of allograft rejection, their role in guiding immunosuppression minimization remains to be defined.

Biology of T suppressor cells

Dr. Wayne Hancock (University of Pennsylvania)

The focus of the discussion was on the epigenetics of naturally occurring T regulatory cells (Tregs). Tregs constitutively express both CD4 and CD25, with FoxP3 as the key regulator of suppressor T-cell function. *In vitro* studies (6–8) suggest that Treg function is modulated by acetylation/deacetylation of Foxp3 and histones. Generally, acetylation of histone tails by histone acetyltransferases (HATs) promotes a permissive remodeling, and deacetylation by histone deacetylases (HDACs) promotes a repressive chromatin state (9–14). *In vivo* studies show that HDAC inhibitors used together with sirolimus induced permanent, Treg-dependent cardiac and islet allograft survival, and donor-specific allograft tolerance (8). Since calcineurin inhibitors depress Treg function through decreased Foxp3 binding to target genes, Dr. Hancock hypothesized that the present dependence on calcineurin inhibitors as primary immunosuppression might impair development of tolerance. While the work described here used animal models, there is reason to think that the findings are applicable to humans since the percentages of Foxp3+ CD4+ CD25+ T cells in calcineurin inhibitor (CNI)-treated solid organ transplant recipients were significantly lower than in healthy controls, and levels of Foxp3+ Tregs were particularly depressed in patients with high CNI levels (15,16).

Immunosuppression: practices and potential for withdrawal

Presenter: Dr. Sandy Feng (University of California, San Francisco)

The type and dosing of calcineurin inhibitors, and the types of adjuvant immunosuppressive medications have shifted. In 1995, 42% and 58% of pediatric recipients were on cyclosporine and tacrolimus, respectively, compared to 4% and 92%, respectively, in 2005 (17). The percentage of patients on azathoprine has decreased from approximately 50% in 1997 to 6.2% in 2004 while the use of mycophenolate has increased from 3.9% to 28%. Corticosteroid avoidance remains uncommon as more than 80% of patients were still discharged on steroids in 2005 (17). Less than 10% of patients treated with cyclosporine were on monotherapy at 24 months after transplantation. In contrast, over 50% of patients transplanted in 2001–2003 who received tacrolimus were on monotherapy 2 years after transplantation. Both total daily and weight-based doses of cyclosporine and tacrolimus have progressively declined.

Single centers have reported functional tolerance in populations of liver transplant recipients (18–21). At the University of Pittsburgh immunosuppression withdrawal was attempted in 98 adult and pediatric LT recipients (18). Overall, 19% were successfully weaned, 39% were still weaning, 29% developed rejection and an additional 19% remained on immunosuppression and were no longer weaning. At Kyoto University, 87 (15%) of their entire, unselected cohort of 581 pediatric living donor liver recipients have been weaned off of immunosuppression (19,21). Acute rejection episodes occurring during controlled, closely supervised immunosuppression withdrawal have been mild to moderate in histologic severity and reversible (18–21), almost never requiring antilymphocyte antibody treatment. Moreover, graft loss related to immunosuppression withdrawal was not observed. These observations suggest that a subgroup of pediatric LT recipients can be safely withdrawn from immunosuppression although the phenotype of this subgroup remains undefined.

Session 3: Long-Term Medical Complications

Posttransplant renal disease

Presenter Dr. John Bucuvalas (Cincinnati Children's Hospital)

The incidence of renal insufficiency and end-stage renal disease (ESRD) in adults increases with time following LT, with a cumulative incidence of ESRD as high as 10% by 10 years posttransplantation (22–25). In a number of open-label studies, treatment with mycophenolate and decreased use of calcineurin inhibitors was associated with increased GFR (26–28). Most studies of renal function in children have been hampered by the limitations and biases associated with small populations, single centers and insensitive outcome measures. One single center study using measured glomerular filtration rate (mGFR) detected renal insufficiency in 32% of long-term survivors. Independent predictors of renal insufficiency were mGFR at one-year posttransplant and increased time since transplant (29). Preliminary results of a multicenter cross-sectional study of posttransplant renal function using the SPLIT Registry showed that 28.4% of patients had a decreased mGFR. Transplant center, age at transplant, calculated GFR at transplantation and 12 months post-LT, cyclosporine immunosuppression and early post-LT renal complications were associated with decreased mGFR. Models of posttransplant renal function should include time since transplantation, calcineurin inhibitor exposure, comorbidities such as hypertension and factors related to phenotype (underlying renal disease, gene polymorphisms related to calcineurin inhibitor pharmacodynamics)(26,29–32).

Risk, treatment strategies and types of posttransplant malignancies

Presenter: Dr. Thomas Gross (The Ohio State University)

Cancer may occur through donor transmission, recurrence of preexisting cancer or *de novo* cancer following

transplantation. Donor transmitted cancer is rare and may result from transmission of occult cancers from living or deceased donors (33,34). Undetected cancer is present in 1–2% of donors and the estimated risk of transmission to adult liver transplant recipients is 0.3–12%.

Hepatoblastoma and hepatocellular carcinoma account for most transplants for primary tumors. For patients with multilobar hepatoblastoma, the International Childhood Liver Tumor Strategy Group and others have observed that outcome is better if transplantation is done at the time of primary tumor resection (80% 5-year survival) rather than extensive resection with rescue transplantation if tumor recurs (30% 5-year survival) (35). The impact of posttransplantation chemotherapy on outcome remains to be determined. Pretransplant chemotherapy likely increases the risk for posttransplant renal insufficiency and hearing loss (29,36). For patients with hepatocellular carcinoma, complete surgical resection with or without transplantation is the only chance for cure (37).

PTLD (80%) is the most common type of posttransplant *de novo* cancer among children. (30–32). PTLD was usually Epstein–Barr virus (EBV) driven, occurring within 3 years of transplantation. Increased EBV viral load in combination with diminished T-cell function increase risk of early PTLD (38–40). Reduction of immunosuppression in response to increased EBV PCR levels reduces the risk of PTLD (41). Even with established PTLD, reduction of immunosuppression was an effective treatment in about two-thirds of patients. Reduction of immunosuppression combined with rituximab may further increase the chance of remission in patients with CD20 positive PTLD (41,42). In patients with progressive PTLD, treatment with corticosteroids and low-dose cyclophosphamide resulted in a 2-year survival rate of 73% and a 2-year event-free survival of 67% (43). EBV negative or T-cell PTLD had worse prognosis and commonly occurred 5 years or more after transplantation. While the risk of skin cancer in LT is well established (44–46), the risk factors and screening guidelines have not been established for pediatric transplant recipients.

Cardiovascular risk in liver transplant recipients

Presenter: Dr. Elaine Urbina (Cincinnati Children's Hospital)

In the general population, cardiovascular organ damage has been demonstrated decades before clinical outcomes became apparent and the presence of target organ damage correlated with known risk factors including obesity, hypertension, dyslipidemia and insulin resistance (47,48). Current immunosuppressive medications are associated with an increased risk for diabetes, dyslipidemia, hypertension, renal disease and obesity (49–54). Linking risk factors for cardiovascular disease to abnormalities in intermediate endpoints such as arterial stiffness and carotid intima-media thickness may permit focused interventions in high-risk patients before development of clinical disease (55,56). Since lifestyle modification can be effective in reducing risk factors, and pharmacologic therapies are proven in reversing target organ damage, early evaluation and treatment is essential to ensure the best outcomes.

Session 4: Patient Wellness Issues after Liver Transplantation

Growth, cognition and family function

Presenter: Dr. Estella Alonso (Northwestern University)

Most children awaiting LT have growth failure and display catch-up growth 6–12 months following transplantation (57–59). Weight normalizes during the first posttransplant year, but linear growth is delayed well into long-term follow-up with the height distribution of this population consistently lower than normal children (59). A multivariate analysis of growth impairment at 24 months posttransplant showed that height and weight at transplant, steroid exposure beyond 18 months, primary liver disease, posttransplant diabetes, single parent household and transplant prior to 2001 all independently predicted growth impairment (Alonso, personal communication). Recent analyses of older children have found that 10–15% have significant mental disability (IQ$\leq$ 70) (60–62). Learning disabilities may be more prevalent than in the general population as one study demonstrated a discrepancy between IQ and academic performance in 26% (60–62). Family function assessed using the family assessment device (FAD) found that 25% of transplant families scored within the unhealthy range on one of the seven dimensions measured as compared to 19–36% of families reported in FAD validation studies (63). Univariate analysis demonstrated that parental education and employment were more strongly associated with family function than with medical outcomes.

Transition of adolescents to adult care

Presenter: Dr. Debra Lotstein (University of California, Los Angeles)

To maximize long-term health outcomes for pediatric LT recipients, stakeholders must consider the changing needs of adolescents transitioning through early adulthood (64–68). Given the focus on transplant-related health issues, primary care needs are often overlooked including counseling on birth control, sexually transmitted diseases, smoking and drug use. Moreover, transplant hepatologists are unlikely to have expertise in areas core to adolescent health issues. The key focuses for a successful transition program include (1) changing needs in health care, including transferring care to adult specialists and primary care providers; (2) changing sources of health insurance; (3) development of decision making and disease

self-management skills and (4) planning for educational and vocational opportunities (69–71).

Health-related quality of life (HRQL)
Presenter: Dr. Jayant Talwalkar (Mayo Clinic)

With improvements in long-term survival, outcomes following LT have expanded to include HRQL (72). Before 2000, most reports on HRQL in pediatric liver recipients were cross-sectional investigations employing a variety of generic and transplant-specific instruments with varying degrees of validity. However, recent studies using validated instruments have demonstrated HRQL ratings for pediatric LT recipients as comparable or better than individuals with chronic disease but lower than healthy children (73,74). The major domains responsible for reduced or improved HRQL are physical and psychosocial function. Factors associated with improved HRQL included younger age at transplantation, longer time elapsed since transplantation, higher level of maternal education and Caucasian race.

Adherence among transplant recipients
Dr. Eyal Shemesh (Children's Hospital of Philadelphia)

A metaanalysis concluded that 23% of adult transplant recipients would be nonadherent to the immunosuppressant regimen in the course of 1 year (75). The consequences of nonadherence are significant and include graft rejection and death (75–79). Yet, although nonadherence is common in pediatric LT recipients and may be the most important predictor of late allograft rejection (77,79), it has not been studied across centers. Without a valid and objective method with defined thresholds to assess adherence, it is difficult to proceed with clinical applications and evaluate interventions to improve adherence. Based on the linkage between whole blood tacrolimus levels and specific graft-related outcomes, there is a unique opportunity to identify and confront medication nonadherence (77,79). A systematic approach could be employed to identify acceptable ranges and standard deviations for tacrolimus levels, with values outside these parameters signaling nonadherence.

Meeting Summary and Recommendations for Future Research

Long-term immune suppression is related to a number of complications in pediatric liver transplant recipients. Current practice consists of dosing and measuring calcineurin levels as an indicator of the degree of immune suppression. However, drug levels do not correlate directly with the extent of immune suppression and consequently may expose individuals to either over or under suppression. Identification of biomarkers that predict risk and the adequacy of immunosuppression would lessen the potential for exposure to immunosuppressant medications and potentially identify patients for immunosuppression minimization and/or withdrawal. Additionally, exploiting current understanding of immunologic mechanisms of tolerance such as the role of regulatory T cells presents as an opportunity that may lead to decreased exposure to immunosuppressants. Changes in a child's T-cell repertoire with age may be of particular relevance for alloimmunity given the potential for functional tolerance noted in pediatric liver transplant recipients. Late allograft dysfunction is an emerging problem for which we do not understand the prevalence, pathogenesis, outcome or treatment. The factors that predispose to increased risk for the development of medical complications such as renal insufficiency and malignancies remain incompletely understood. Once risk factors are identified, intervention studies would assess risk reduction to minimize immunosuppression side effects and individualize immunosuppressive therapy.

Current qualities of life measures are generic and do not adequately reflect the distinct clinical issues that confront pediatric liver transplant recipients. Development of instruments that would incorporate both objective liver related measures coupled with quantification of physical activity status such as school attendance or occupational performance or level of activities of daily living into an index or scale would serve as a measure of a spectrum of functional outcomes for pediatric liver transplant recipients. Such an index would facilitate future outcome studies in this patient population.

Risk-taking behaviors and nonadherence to medical regimens are prevalent among adolescents and may influence the health and well-being of liver transplant recipients during transition. Moreover, the data presented here suggest that late allograft loss may be in part related to nonadherence. Additionally, adolescence is associated with significant physical and intellectual growths that are hindered by liver transplantation. Furthermore, the transition of liver-transplanted children to adulthood poses additional challenges that impinge upon long-term patient well-being and graft survival.

In conclusion, the important issues that compromise the long-term well-being, health and survival but are amenable to be addressed with existing and new research efforts include:

- Longitudinal assessment of outcomes for pediatric liver transplant recipients so as to permit the recognition of emerging trends of clinical challenges as the cohort ages. The effort would be enhanced with consistent and uniform approaches to data and biologic specimen collection. Utilization of well-characterized long-term patient cohorts which include tracking patients into early adulthood analogous to what has been done for cancer survivors provides a vehicle for research into identifying barriers and facilitators which in turn, will promote the development of interventions

designed to maximize health-related quality of life and adherence.
- Identification of risk factors associated with long-term immunosuppression complications such as renal failure, malignancy, infections and cardiovascular complications. By doing so, we may stratify patients based on their risk and ultimately develop interventions (1) to prevent disease, (2) prevent progression of disease and (3) to salvage function.
- Determine issues and risk factors associated with late graft dysfunction and loss with particular focus on late biliary and vascular complications, an emerging cause for late graft loss and dysfunction.
- Develop tolerance-inducing regimens based upon currently known immunologic molecular mechanisms.
- Define biomarkers that accurately reflect the level of clinical immunosuppression which can then be employed to individualize immunosuppression regimens including the systematic withdrawal of immunosuppression in selected patients.
- Develop accurate and specific measures of wellness that encompass the unique issues associated with pediatric LT recipients are needed.
- Identify risk factors that impede growth and intellectual development prior to and after liver transplantation.
- Identify barriers and facilitators of risk taking and nonadherence behaviors during adolescence as a foundation for directed intervention efforts.
- Advance our understanding of how to maximize health-related quality of life and adherence among adolescents and provide effective health care transition programs.

At the present time, these issues are poised to be addressed and can yield valuable information that would be directly translatable to improved clinical care for pediatric liver transplant recipients.

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