Graft Fibrosis in Stable Pediatric Liver Transplant Recipients: What Does It Mean?

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Liver transplantation offers hope for those in need. This hope is perhaps most palpable in the pediatric population, where the chance for a full lifetime of opportunities is approaching reality. Significant improvement has been made in short-term survival, and 1-year patient survival in most pediatric age ranges is now above 90%.[1] Additionally, patient survival after the first year remains relatively constant for several years. This success has permitted the opportunity to shift efforts toward evaluating long-term outcomes,[2] with a growing focus on improving overall long-term health and issues related to chronic immunosuppression.[3,4] On the surface, all is reasonably well; in terms of clinical and biochemical measures, most recipients appear to have good graft function.

In this issue of HEPATOLOGY, Scheenstra and colleagues report on protocol biopsies in pediatric recipients 10 years after transplantation.[5] This group had previously reported evidence of portal fibrosis in 31% of protocol biopsies (26 of 84) at 1 year following transplantation.[6] The current report extends these observations, following a cohort of 77 children, after excluding seven of the original subjects who at 1 year had evidence or rejection, viral infection, or vascular changes. They report the prevalence of fibrosis increased to 65% at 5 years after transplant, and 69% at 10 years. Although the prevalence did not increase after 5 years, the percentage with severe fibrosis increased from 10% at 5 years to 29% at 10 years. Of further concern, of those with no evidence of fibrosis at 1 year after transplant, 64% had fibrosis at 5 years after transplant. The fibrosis was in children who appeared clinically stable. While there was a trend for higher liver function tests (LFTs) at time of biopsy with fibrosis, with an elevated γ-glutamyl transferase level being the most consistent finding, there was considerable variation with a wide overlap of ranges between those with severe fibrosis and those with no fibrosis. Most had either minimally elevated or normal LFTs. Fibrosis was associated with younger recipient age at transplant, higher donor/recipient age ratio, longer cold ischemia times, and use of partial grafts.

Others have reported unsuspected pathology on protocol biopsies in the setting of normal liver function tests.[7] Much of this literature is in the adult population, where recurrent disease is common and may confound evaluation of this issue.[8,9] In the pediatric population, there have been several reports of unsuspected pathology increasing with time. Rosenthal and colleagues found mild fibrosis in 8% of protocol biopsies at 3 years after transplant despite normal LFTs.[10] In a study of children with biliary atresia who underwent transplant, Fouquet et al. reported abnormal histology, primarily chronic rejection or centrilobular fibrosis, in 73% of recipients 10 years after transplantation.[11] Again, all these children had normal or near normal LFTs. Evans et al. reported chronic hepatitis in 64% of biopsies at 10 years, with fibrosis common. In 15% of the cases, the fibrosis was graded as severe.[12] Fibrosis was strongly associated with presence of autoantibodies and findings of chronic hepatitis defined by a predominantly portal-based mononuclear inflammation. The biliary or vascular changes characteristic of chronic rejection were minimal or absent. The incidence of fibrosis also increased over time, suggesting a progressive injury, and it was speculated the hepatitis represented some form of rejection.

Does the progressive fibrosis observed by Scheenstra and the others represent some form of chronic rejection? While the incidence of chronic rejection today is low,[13] and there is no mention of either the bile duct injury classically associated with chronic rejection or chronic hepatitis, is it reasonable to assume we have nearly eliminated the mechanism or that the liver is entirely resistant to chronic insult? It is possible the fibrosis reflects an alloimmune process. The role of alloimmunity on graft fibrosis has been perhaps most extensively studied in kidney transplantation, where fibrosis is a leading cause of graft loss.[14] In the kidney, the process previously referred to as chronic rejection appears to be the result of antigen-dependent and antigen-independent mechanisms. With respect to the current study, increased ischemic times are also associated with kidney graft fibrosis, a finding believed to reflect interaction with ischemia reperfusion, the innate immune system, and the alloimmune response. However, in contrast to kidney transplantation, preserva-

Abbreviations: LFT, liver function test; TGF-β, transforming growth factor-beta.

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tion injury does not appear to increase risk or severity of acute rejection in liver transplantation.[15] In kidney transplantation, acute rejection is a strong risk factor for subsequent fibrosis. In the current study, acute rejection was not associated with fibrosis. In fact, this group previously reported that rejection appeared protective from fibrosis at 1 year after transplant.[6] Nonetheless, it would be worthwhile to look for evidence of an increased alloimmune response in patients with fibrosis, such as donor-specific antibody or via functional T cell assays. Whereas more immunosuppression typically has no impact on chronic rejection, it is clear insufficient immunosuppression is a risk factor. Importantly, the pediatric population is transitioning from childhood to adulthood, and adolescence is a well-established risk factor for nonadherence.[16] Insufficient immunosuppression by nonadherence could allow chronic low-grade injury to manifest as fibrosis. With respect to immunosuppression, most in this series initially received cyclosporin, prednisolone, and azathioprine, with 44 recipients having had cyclosporine withdrawn after 2 years. Fibrosis was not associated with withdrawal of calcineurin inhibitors or the immunosuppression regimen, potentially suggesting this process is not sensitive to the amount of immunosuppression. Currently in the United States approximately 90% of pediatric recipients receive tacrolimus.[17] Will this affect the risk of fibrosis going forward? Given that less immunosuppression does not lead to more fibrosis, it is difficult to envision less risk with more immunosuppression, especially absent an impact of acute rejection.

Although alloimmune processes could contribute to this progressive fibrosis, other mechanisms should be considered. The liver appears relatively resistant to such injury, and perhaps the underlying mechanisms are responsible for the fibrosis. The liver has a wide range of cell types known to have immunoregulatory capabilities.[18] There has been growing evidence regarding the role of regulatory cells. Regulatory/suppressor T cells include several T cell subtypes such as CD4+CD25+ regulatory T cells.[19,20] Regulatory cells exert some or all of their immunoregulatory effects via release of cytokines, including interleukin-10[21] and transforming growth factor-beta (TGF-β).[22] These mechanisms may generate effective regulation and render the liver less susceptible to alloimmune injury, but a consequence of this localized relative overproduction of these specific cytokines could be fibrosis. An example of how such a mechanism could be pathologic is immunoglobulin G4–related sclerosing pancreatitis and cholangitis, an autoimmune process associated with progressive fibrosis, where there appears to be a relative overabundance of T lymphocytes with a regulatory phenotype (CD4+CD25+Foxp3+), along with relative overexpression of messenger RNA of regulatory cytokines (including TGF-β).[23]

The biology of liver transplantation is unique in that a small, select proportion of recipients can be withdrawn from immunosuppression and not develop rejection. At present, there are no reliable markers to predict a priori which patients can successfully be withdrawn, and it is not a routine clinical practice. Nonetheless, this observation offers a unique opportunity to further our understanding. This state of operational tolerance appears to involve nondeletional mechanisms via regulatory T cells. If graft fibrosis is caused as a bystander effect from regulatory mechanisms, and assuming the same mechanisms are involved after successful withdrawal of immunosuppression, one might expect more fibrosis in such recipients compared to recipients on immunosuppression. Interestingly, biopsies from pediatric living donor recipients successfully weaned from immunosuppression have been preliminarily reported to have more fibrosis than those maintained on immunosuppression.[24]

The impact of age on the risk for fibrosis is also intriguing. Several mechanisms may be responsible. As mentioned, adolescents are at increased risk for nonadherence to immunosuppression. As children mature, their immune system changes. Infants lose their relative immunologic immaturity and become more adept at mounting immune responses. In liver transplantation, infants have a lower rate of acute rejection compared to older children, and this finding is associated with a Th2 phenotype.[25] Although this pattern of immunoregulation may help minimize alloreactivity in the short term, the Th2 phenotype can also be associated with chronic rejection and fibrosis. The proportion of T cells with a memory phenotype also increases with age. Memory T cells have lower activation thresholds and appear to be more difficult to regulate.[26] It is likely that both age at transplant and the child's current age profoundly affect the state of the immune system.

Aside from the potential role of immunologic factors, there are other mechanisms which may be operative. The risk factors identified by the authors (younger age, partial grafts, and longer ischemic times) may indicate a more difficult operation, with increased risk for technical issues. Perhaps the fibrosis reflects subclinical biliary or venous outflow obstruction which eventually manifests. In addition, age-dependent variation in exposure or susceptibility to substances in the portal circulation (e.g., endotoxin) or other environmental factors may create a profibrotic milieu. Finally, in contrast to adults, the graft needs to grow with the child. Perhaps this physiologic growth, in the unique setting of the transplant recipient (e.g.,

chronic immunosuppression, alloimmunity) results in fibrosis.

What should be done clinically given these findings? A low threshold for biopsy for cause is appropriate. Approximately one-third of children surviving 5 years after transplant do not have normal aminotransferase levels at their 5-year anniversary visit.[4] Minimal chronic elevation in aminotransferase levels may reflect significant ongoing injury, and biopsy is warranted. In those with normal LFTs, the role for protocol biopsies is more difficult to advance absent a better understanding of the mechanisms responsible for the process. Even with better understanding, an effective intervention would be necessary. Protocol biopsies are clearly essential in the context of a clinical trial, or planned immunosuppression withdrawal, because one will not know whether the intervention is associated with progression or minimization of fibrosis.

In summary, the report from Scheenstra et al. demonstrates that despite ideal outcomes, fibrosis can develop in grafts over time. The progressive increase in both the prevalence as well as the severity of fibrosis is of concern, and relevant to both pediatric and adult recipients. Whether this fibrosis will lead to clinical graft dysfunction or significant rates of graft loss in the future is unclear. Until we have a mechanistic understanding of the process, our ability to offer a prognosis or intervene is limited. Retrospective studies or single-center trials in the pediatric population will not provide sufficient insight. Well-designed, multicenter prospective studies are necessary to understand the long-term impact of fibrosis, the potential role of protocol biopsies, and the underlying biology.

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