Published in final edited form as:

J Pediatr Gastroenterol Nutr. 2008 March ; 46(3): 238–240. doi:10.1097/MPG.0b013e318163357a.

Swiss outcomes in Biliary Atresia: Are there lessons to be learned?

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Perhaps the greatest challenge in pediatric hepatology is to understand the etiology and pathogenetic mechanisms underlying biliary atresia (BA), a unique dynamic, inflammatory fibroobliteration of the extrahepatic and intrahepatic bile ducts that occurs exclusively in the first few months of life. Virtually no other childhood liver disorder produces equivalent morbidity and mortality without liver transplantation, yet advances in treatment of BA have been hampered by limited knowledge of its pathogenesis. It has long been known that performance of the Kasai portoenterostomy at a younger age confers a better chance for successful post-operative bile drainage, normalization of serum bilirubin and the possibility for long-term survival. But why? We also now know that there is a fourfold-variance in the incidence of BA across the world, but without a good explanation. Several discoveries in recent years have begun to shed light on these and other issues in this enigmatic ailment.

There appear to be several disease processes that can generate the BA phenotype¹, including 1) defects of bile duct morphogenesis (the so-called *embryonal* form; 20% of cases), both with and without other congenital malformations, and 2) an inflammatory dynamic cholangiopathy caused by a presumed pre- or post-natal insult (the *perinatal* or acquired form; 80% of cases) which leads to progressive sclerosis and bile duct obstruction during postnatal life. Several candidate genes that regulate bile duct and liver differentiation and development may be involved in the genesis of the embryonal form of BA², although no clear association has yet been discovered. One theory holds that physical damage of the developing extrahepatic bile duct, whatever the underlying etiology, may result from disrupted bile duct epithelia with subsequent extravasation of bile with its detergent constituents into the submucosa, eliciting a secondary inflammatory sclerosing process. In this regard, Tan et al.³ have observed extravasated bile in bile duct remnants of BA patients. This group further proposed that there was a vulnerable stage of human biliary development between 11 and 13 weeks of gestation, when failure of remodeling of the ductal plate structures could impair the formation of an adequate mesenchymal cuff around developing hilar bile³ ducts. Upon initiation of bile flow at 12 to 13 weeks of gestation, these ducts could potentially be prone to rupture, leaking bile into adjacent periductal tissues that would undergo protracted inflammation and fibrosis, culminating in secondary obstruction of the more distal extrahepatic bile duct.

The more common perinatal form of BA is believed by most to be caused by a viral-induced immune response that primarily causes bile duct epithelial apoptosis, uncovering neoantigens that elicit a secondary immune response, setting up a persisting inflammatory state that leads to further tissue injury and fibrosis of the bile duct. Because of the work of

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Disclosure: Dr. Sokol is an investigator and Chair of the Steering Committee of the Biliary Atresia Research Consortium, and is a member of the Scientific Advisory Board of Yasoo Health, Inc.

Riepenhoff-Talty⁴, Petersen⁵, and others, a well characterized viral-induced (Rhesus rotavirus; RRV) mouse model for BA is now available that bears considerable anatomic, histologic, and immunologic resemblance to the perinatal form of the human disease. Unfortunately, definitive evidence of a viral trigger in infants with BA has not been confirmed. Indeed, rotavirus, reovirus, cytomegalovirus, and other viruses have been implicated in infants with BA by some studies⁶⁻⁹, but not confirmed by all.¹⁰⁻¹² One explanation is that viral clearance occurred before the infants presented with BA confounding detection of viral nucleic acids, as has been demonstrated by 2-3 weeks of life in the RRV-infected mouse model.^{13, 14} Newer translational approaches will clearly need to be utilized in order to bring the question of a viral etiology of BA to a close, and whether immunization, antiviral therapy, or other approaches could be tested to prevent, identify or treat this disease.

In recent years, immune and autoimmune pathways of bile duct injury in BA have been elucidated in both the mouse model and infants with BA. Indeed, both a Th1 CD 4 and CD8 T-cell immune response are directed towards bile duct epithelia^{13, 15}, both in the RRV-infected infant mouse model and in the human infant with BA. Activation of the innate immune system also appears to be involved as demonstrated in a recent study which reports upregulation of Toll-like receptor (TLR)-7 and high expression of MxA, a protein downstream of signaling by TLR-7, in the early stages of human BA.¹⁶ Moreover, the macrophage migration inhibitory factor 173G/C polymorphism was significantly associated with BA (OR = 6 [95% CI 2.8-11.5], $p < 0.0001$) in 18 patients from Turkey, suggesting that this might be a susceptibility gene for BA. The role of innate immunity in the pathogenesis of BA is an exciting area for future investigation.

Further evidence of immune dysregulation playing a major role is the observed oligoclonal expansion of T-cells in liver of infants with BA characteristic of an immune response targeted towards specific antigens.¹⁷ Moreover, adoptive transfer experiments demonstrated that bile duct injury was recapitulated in immune-deficient mice receiving hepatic and splenic T-cells from RRV-infected infant mice with BA.^{15, 18} Thus, defining specific inflammatory pathways involved in autoimmunity that may be interrupted poses yet another potential treatment strategy that has yet to be fully exploited.

In parallel with this renewed interest in defining the etiology and pathogenesis of BA has come a clearer assessment of clinical outcomes and factors predicting survival with native liver (SNL), thus avoiding or delaying the need for liver transplantation. In the French national studies of Chardot and colleagues¹⁹ which examined 439 and 253 patients with BA over two recent time periods, four factors were demonstrated to have a significant negative impact on SNL: the anatomic subtype of atresia, the presence of polysplenia syndrome (BA splenic malformation syndrome), a lower level of surgical experience at the center performing the portoenterostomy, and age over 45 days of age compared to < 45 days. In other studies, the presence of cirrhosis at diagnosis²⁰ and episodes or ascending cholangitis were also predictors of poor outcome. The BA Research Consortium in the United States reported that serum total bilirubin level at 6 months following portoenterostomy served as a highly predictive biomarker of SNL at 2 years of age.²¹

In this issue of the Journal, Wildhaber et al.²² present a thorough evaluation of the BA experience in Switzerland over a recent 11-year period. Data were gathered from all 7 Swiss centers in which BA surgery is performed, yielding an annual incidence of BA of 1 in 17,800 live births (95% CI: 1/13,900 to 1/24,800). This compares favorably with 5 prior European studies (reviewed by Wildhaber et al.²²) which yielded a composite incidence of 1/18,400. In contrast, three studies from the United States reported incidences of 1/11,800 to 1/15,400 and studies in Asia report 1/2700 (Taiwan) to 1/11,200 (French Polynesia).

Clearly, there is a substantially higher incidence of BA among Asians and even Americans compared to Europeans. How do we explain this and does this bear on possible pathogenesis of BA? The simplest explanation is that there must be genetic or environmental differences related to BA incidence, if we assume that the disease is being diagnosed correctly in all countries and that the BA phenotype is identifiable. An as yet uncharacterized genetic predisposition to BA may be more common amongst certain populations or an environmental factor (such as a perinatal viral infection) may be more active in crowded living conditions or in certain climates. Thus, the higher incidence of BA in Asia is clearly a clue to its etiology that must be actively pursued, perhaps through high throughput genomic or genetic studies, screening for biomarkers of viral or toxin-mediated disease, or comparative epidemiologic investigations. We believe that sharing across centers and continents of precious BA registry and database data, stored clinical specimens and banked DNA would take advantage of these naturally occurring differences and allow modern biotechnologic approaches to yield important new answers about BA pathogenesis.

In a similar collaborative manner, combining longitudinal datasets for several thousand BA patients from around the world should provide the statistical power to definitively determine the influence of age at portoenterostomy (and other predictive factors) on surgical outcomes. In the Swiss study, despite a relatively small number of patients, the best SNL was found in those patients operated on prior to 45 days of age, similar to some other reports. The usual dogma states that we should try to diagnose and operate on BA patients prior to 60 days of life, however, the Swiss and the French national studies call this age cut-off into question. Achieving optimal SNL results may mandate surgery prior to 45 days of life. This would pose a particular challenge in the United States where infants are generally not seen by their health care provider between 1-2 weeks of life and 2 months. If indeed this age for best surgical outcomes is confirmed, renewed interest would be generated for some form of newborn or infant screening for BA. Stool color cards appear to be a successful screening tool in Taiwan²³, however the logistics of this program in the United States and some other Western Countries might be challenging. Based on this urgency to determine optimal age for portoenterostomy, there is an unmistakable need for an international effort to develop and analyze this proposed longitudinal international combined BA database and better understand outcomes and natural history following portoenterostomy. Parallel and integrated multi-centered translational investigations have the potential to uncover the etiology of this rare disease by the end of the decade, and change the clinical paradigm to one of prevention and cure. Yes, there are certainly lessons to be learned in this devastating disease!

Acknowledgments

Supported in part by grants from NIH (UO1-DK062453, U54 DK078377 and MO1 RR00069)

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