

Cholestasis: Current Issues and Plan for the Future

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Issues and concepts raised in the previous 2 position papers (1,2) still need to be addressed and will be reiterated here. The first paper focused on biliary atresia and the second on drug-induced cholestasis. The vast difference in focus points to the heart of the matter. Cholestasis represents an enormous group of varied and rare disorders. Unlike some of the other position topics, it is the rare disease that is most likely to benefit from an international effort. The study of rare disorders requires a community of workers, Web sharing tools, and registries to advance the field.

Cholestasis is a sign or symptom and not a disease. It is defined as a pathological state of reduced bile formation or flow (3). This definition applies more to the experimental situation, in which the rates of bile formation and flow can be measured, than to human cholestasis, in which neither can be assessed. Therefore, the clinical definition of cholestasis is any condition in which substances normally excreted into bile are retained. The serum concentrations of conjugated bilirubin and bile salts are most commonly measured. Those affected with cholestasis have a wide range of diseases including rare genetic, metabolic, and infectious liver diseases.

Cholestasis is a clinically significant entity because of its association with significant morbidity and sometimes mortality. Our gaps in knowledge are obvious. We frequently do not understand the cause of the problem and even use terms such as idiopathic neonatal cholestasis. When we do know the cause, we still lack diagnostic methodology and our ability to prevent or intervene is inadequate. As our commitment to the World Congress

grows and our ability to communicate across vast distances improves, it is now the time to develop the infrastructure needed to address global health care.

Developing a collaborative world program for cholestatic liver disease will be helpful to advance the science and clinical care for this collection of diseases. We suggest moving forward in identifying key problems, followed by the larger task of developing a database and Web sharing tools. Four issues will be discussed:

- Understanding the pathogenesis of cholestatic liver disease
- Developing diagnostics
- Developing therapeutics
- Developing an informational Web site and patient registry

CURRENT CONTROVERSIES OR ISSUES

Understanding the Pathogenesis of Cholestatic Liver Disease

Pediatric cholestatic liver disease encompasses a vast array of rare disorders (3). Over the past 2 decades, our ability to diagnose specific conditions has improved and slowly we have chiseled away at the category known as idiopathic neonatal cholestasis (4). We are able to label a number of conditions, but we still do not clearly understand the pathogenesis for most of them. As recently as the 1980s, 40% of cholestatic infants were labeled as having idiopathic neonatal cholestasis. In 2007, the category has been reduced to 20% or less. New understanding of cholestasis has grown to include mitochondrial disorders (5), bile transporters (6), *JAG* and *NOTCH* deletions (7–9), among others. However, even with this newly gained knowledge, there is still a need for

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a deeper understanding. From knowledge, we will be able to improve upon screening programs and interventions.

Pathogenesis has 2 features. The first is the primary defect, and the second includes the associated factors accounting for patient phenotype. α -1-Antitrypsin deficiency can serve as an example (10). The primary diagnosis is straightforward and can be made by Pi typing. However, Pi type only predicts a limited amount with regard to phenotype. A number of other genetic and environmental factors influence pathogenesis. Ultimately, it is important to understand both primary and secondary factors in pathogenesis to predict outcomes and develop disease-specific interventions.

Guidelines to Improve Our Understanding of Pathogenesis

- A. Identify the primary defect(s) accounting for the still large set of idiopathic causes of neonatal hepatitis
- B. Identify primary defect and secondary defect for diseases that are described by phenotype (eg, biliary atresia)
- C. Identify secondary defects for diseases such as Alagille syndrome, α -1-antitrypsin deficiency, and progressive familial intrahepatic cholestasis, in which the primary cause is known but the secondary defects are still lacking

Similar techniques can be used for the study of A, B, and C stated above. New methodologies are ripe for being applied to the study of cholestatic liver disease. Genome-wide screening (11), proteomics, metabolomics, lipidomics, and microarray analysis of viral sequences (12,13) all are promising avenues that should be explored and have proven useful in other systems. These methods use an open-ended approach. Genomewide screening can quickly and analytically compare sequences among large groups simultaneously. The DNA is compared with known areas of sequence variability. If constructed correctly with the appropriate clinical information, new genetic diagnoses can be made. This has been useful recently in identifying the genetic basis of macular degeneration and in identifying the interleukin-23 receptor as a potential contributor in inflammatory bowel disease (11,14). Proteomics, metabolomics, and lipidomics also may aid in the identification of biomarkers involved in the pathogenesis of cholestasis, especially when combined with appropriate controls and clinical information for analysis. For many of the cholestatic conditions, it is thought there are environmental triggers that have not been identified. Again, applying new methodologies will prove useful. Microarray technology using viral sequences has been useful to identify multiple viruses at once. If done with short viral sequences, it is possible with a number of steps to even construct the

sequence of novel viruses, as was done for the severe acute respiratory syndrome virus (13). In each case, what is needed is adequate sample size, appropriate tissue, genetics data, and clinical information.

Developing Diagnostic Tests

Diagnosing many of the cholestatic conditions is difficult. Often, there is a need for detailed and esoteric knowledge to arrive at the correct diagnosis. Physical findings are subtle. Infants often look healthy; the only finding may be a yellow tinge to the eyes. Furthermore, the development of these findings may not coincide with a routine pediatrician examination. The tests often require large blood or urine volumes shipped to far reaches of the world, then tested in unregulated laboratories. Because timeliness significantly affects outcome for many diagnoses, it is necessary to develop early efficient screening programs. There is a need for tests that can be performed easily even for those practitioners who are less knowledgeable. Furthermore, the test should not be an excess burden to the patient. Given this set of circumstances, the steps needed to improve the field of cholestasis are outlined in the following paragraphs.

Guidelines to Promote Ease of Timely Diagnosis

- A. Early screening programs
- B. Diagnostic tests based on presentation
- C. Disease-specific tests
- D. Ease of finding information

Early Screening Programs. Screening programs have gained much interest. The most commonly discussed are the stool color cards that have been piloted in a number of countries (15,16). The preliminary data has shown that the cards can help alert parents and providers to a child who may have biliary atresia or another biliary issue. They are easy to use, inexpensive, and can be interpreted without the need for a skilled nurse. Parents can be instructed to send the cards by mail to the physician's office. In the United States, this may be particularly useful, because the current recommendations for well-baby visits are at 2 and 8 weeks of age. Such timing may be too early and too late for a diagnosis such as biliary atresia. A 4-week check for acholic stools can be useful. Other approaches that can be taken are to educate parents that a baby's urine should appear colorless in the diaper (16). This is a simple and often overlooked fact. More scientifically, a screening program piloted in England examined direct bilirubin in the neonatal period and was done as a home visit (17). This last approach casts a wide net and can pick up a variety of neonatal liver pathologies. Other general

screening mechanisms have been proposed, as well (18,19). Each of these approaches represents a slight variation in tackling early and convenient diagnosis of neonatal liver pathology.

Diagnostic Tests Based on Presentation. As stated above, many diagnoses require esoteric knowledge. To simplify diagnostics, it would be extremely valuable to develop tests that assay for a number of problems simultaneously and require a small volume of either blood or urine. Such tests are in development using gene chip technology. To date, the tests have focused on genetic cholestatic diseases (20). Expansion upon this concept should be encouraged. Viral disorders have not been included, yet for many of the early viral hepatopathies the virus is found in blood or urine and the genetic sequence is known. Further development of this concept is simple.

Disease-specific Tests. The largest stumbling blocks are those diagnoses that are established exclusively by phenotype, such as biliary atresia and idiopathic neonatal hepatitis. We still use a multistep process of invasive and time-consuming tests to arrive at a final diagnosis. Newer diagnostics will result from a better understanding of pathogenesis; thus, it is an essential first step to clarify the pathogenesis.

Ease of Finding Information. The last issue to be discussed regarding diagnostics is the common problem of finding accurate, comprehensive, and up-to-date information. The best way for this to be handled will be to develop a single source of web information that is overseen by the organizations comprising the World Congress. This site should be updated regularly and include information on laboratories currently performing these tests and indications for when the test should be performed.

Identifying Therapeutic Agents That Will Improve Outcomes in Chronic Cholestatic Liver Disease

Many aspects of chronic cholestasis impair health. Patients suffer from altered nutrition, osteopenia, and pruritus. Nevertheless, overwhelmingly the most significant health issue is the fibrosis–cirrhosis continuum. Ultimately, the development of cirrhosis is what leads to many significant issues that affect morbidity and mortality. This includes portal hypertension, ascites, coagulopathy, and hypersplenism. Fibrosis is defined as abnormal scarring in the liver. Typically, fibrosis occurs even though the hepatocyte is able to function. As newer knowledge has developed, we now understand that fibrosis and cirrhosis are dynamic states. Neither is permanent. Knowing this, and knowing that many of the liver cells are healthy, it is obvious that curing fibrosis will be significantly advantageous.

Alteration in the extracellular matrix occurs in many cholestatic liver conditions. Unfortunately, this change leads to more change and a vicious cycle ensues resulting in increasing deposition of inappropriate matrix (21). The extracellular matrix is increased approximately 10-fold in the fibrotic compared with the normal liver. Because the extracellular matrix has both structural and signaling functions, these changes have far-reaching effects. In this process, the space of Disse is replaced by a complete and continuous basement membrane. The vascular flow is altered in the sinusoidal space, and the exchange of usual substances and local cell signaling is changed.

In the past decade, the molecular pathways contributing to fibrosis have begun to be elucidated. Our understanding has led to potential targets for therapeutics. The metalloproteinases are responsible for extracellular matrix degradation and remodeling (22,23). Transforming growth factor β is necessary. Other important factors in developing liver fibrosis and cirrhosis include: hepatocyte growth factor, platelet-derived growth factor, bone morphogenic protein-7, hepatic stellate cells, and myofibroblasts (24). Each is a potential target for therapeutics.

Although ultimately most cirrhotic and fibrotic livers look outwardly similar, the truth is they must be more varied than we currently understand. As pediatric gastroenterologists, we have extensive experience with biliary atresia and know that the pace at which cirrhosis develops outstrips that of almost all other liver diseases. Development of cirrhosis in hepatitis C is monitored over decades, whereas in biliary atresia it is measured in months. Serum measures that correlate with staging in hepatitis C are useless in biliary atresia. These differences provide a clue to different metabolic pathways.

It is likely that there will be broad spectrum antifibrosis agents and more specific disease agents. As stated previously, our therapeutic arsenal for chronic cholestasis is inadequate. Most of the measures taken to treat cholestatic conditions are marginally palliative, such as nutritional support, bile acid binding agents, and surgical procedures including hepatopuertoenterostomy and biliary diversion. Intervening in the process of fibrosis, or better yet reversing its development, would be the most critical advancement in the field of chronic cholestatic liver conditions.

Guidelines to Study the Mechanisms of Fibrosis

- A. Identify new factors that effect disease progression in pediatric cholestatic diseases
- B. Test current targets for therapeutics in appropriate disease models, laying the groundwork for human trials

Developing an Informational Web Site and Patient Registry

The world has benefited enormously from the Internet. Sharing information via e-mails, chat rooms, and registries has been useful. Yet the World Congress has not developed a collaborative infrastructure that has shared resources. A survey completed at the start of writing this position statement reiterated our lack of continuity from one congress to the next. It is time that the World Congress develops and oversees a Web site that takes advantage of this powerful tool. If done properly, the site can provide current information that is useful to patients and practitioners. Additionally, portions of the site can serve as the foundation of clinical science. The advantage of such a specific endeavor is to provide a comprehensive approach to a number of gastrointestinal issues and especially for cholestatic liver conditions.

The advantage of collaboration through the Web for rare diseases is particularly strong. For patients with a rare disease, it is often difficult to find correct information. Being guided to such a place is useful, if we are able to provide monitored information. Such a tool is relatively cost effective, considering the number of people with access around the world. For practitioners who rarely see many of these diseases, information can be given based on known diagnoses and algorithms can be developed based on clinical presentation. Patient registries can be developed. Cataloging clinical features will help us arrive at new diagnoses and allow us to understand outcomes based on presentation. For those in which the diagnosis is known, we will be able to gather outcome information to understand the natural history for a variety of diseases. We will be able to identify gaps in our knowledge and gain a deeper understanding of beneficial targets for therapeutics.

Registries and databases will provide the backbone for clinical and translational studies (25,26). We will be able to test screening tools and diagnostic methods, and at its most advanced state provide a mechanism for clinical trials. Ethically, this approach will provide opportunities to patients at distant corners of the globe and will provide oversight by a group that is knowledgeable. We can build on the success of similar endeavors by the World Health Organization, EuroWilson, the Biliary Atresia Research Consortium, and the Cholestatic Liver Disease Consortium.

Guidelines for Developing Web-sharing Tools

- A. Develop a Web site with the latest information regarding specific diseases that includes where tests can be performed and treatment options
- B. Develop diagnostic algorithms based on cholestatic presentation

- C. Develop patient registries for specific cholestatic conditions

RESEARCH AGENDA

The research agenda for cholestasis is an outgrowth of the previous discussions. There is a pressing need to better understand many of the cholestatic liver disorders. Screening programs, prevention programs, and new interventions will develop from this understanding. Among those concepts discussed, 3 areas should be our focus.

Examine the Genetic Basis of Cholestatic Liver Disease Phenotype

At this juncture, our understanding of genetics has drastically changed and should be applied to the field of cholestasis, in which many of the disorders are considered "complex" genetic problems and the patient phenotype is not typically Mendelian. Gene copy number, microRNA, and DNA methylation are all important layers of control in genotypic expression.

Develop Antifibrotic Agents for the Pediatric Population

Fibrosis is the most debilitating complication of chronic liver disease. Fibrosis and scar formation lead to nutritional issues, impaired quality of life, and portal hypertension. Addressing this medical complication would broadly impact the field of chronic cholestasis.

Develop an International Registry for Patients With Rare Liver Disorders

Registries are essential for optimizing clinical and translational research. It is through the sharing of information and tissue samples that new genetic diagnoses will be made. They are also the backbone of clinical trials.

Conclusions

The most important contribution of a World Congress is to encourage the collaborative atmosphere that will ensure advancement in the field of clinical hepatology. Cholestatic liver disorders are among the clinical pathologies most likely to benefit from collaboration. The diseases are rare. Sharing information will be beneficial. Some of these topics have been addressed in previous position papers. The most prominent features lacking from one set of guidelines to the next have been continuity, oversight, and continued momentum. To open lines of communication that are continuously worked upon is essential. A designated group that works collaboratively from one World Congress to the next is the best

way to accomplish the most. These goals hinge on developing a useful web of information and studies. Working together serves to establish priorities, set standards, and encourage critical scrutiny of science and clinical care.

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