Cryptogenic Cirrhosis: An Approach To The Diagnosis In The Era Of Molecular and Genomic Medicine

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Introduction and historical perspective: Cryptogenic cirrhosis(CC) is defined as cases of cirrhosis where the etiology remains uncertain after careful and extensive clinical, laboratory and histologic analysis. The incidence of CC has been steadily declining over the years. Prior to 1965 the CC constituted about 50% of all cases of cirrhosis[1]. In 1965 Hepatitis B virus (HBV) was discovered which subsequently led to its recognition as a major cause of chronic hepatitis and cirrhosis worldwide. While autoimmune hepatitis (AIH) was first described as an important cause of chronic liver disease in 1950, its clinical diagnosis remained erratic. The first diagnostic criteria by an international working group were proposed in 1998 and subsequently modified in 2008, which led to improved diagnosis of AIH. Another landmark discovery in this field was discovery of hepatitis C virus (HCV) in 1989, which eventually led its recognition as one of the major causes of cirrhosis worldwide. Non-alcoholic steatohepatitis (NASH) was first described as a clinical entity by Ludwig in 1980, but it took few decades to understand its real impact on the burden of liver disease and contribution to cirrhosis. Now non-alcoholic fatty liver disease (NAFLD/NASH) has been recognized as the next big epidemic in chronic liver disorders. Over these years better understanding of many other metabolic disorders and their improved diagnosis [Wilson's disease, A1AT deficiency, familial inherited cholestatic (PFIC) syndromes, iron overload syndromes] also contributed to the continued decline of CC.
Currently CC seems to accounts for an estimated 5-30% of cases of cirrhosis in various studies[1]. The wide difference in the reported incidence likely results from variations in clinical practice and resources available at different Institutions across the world. Some of it may also be contributed by ethnic and geographic differences[2,3]. Overall, in developed countries and tertiary care medical centers, CC accounts for about 5-10% of all cases of cirrhosis. It accounts for about 10% of liver transplants and is present in 3-14% of adults awaiting liver transplantation. As the diagnosis of liver disorders continues to evolve, the proportion of CC cases continues to decline.

Clinical implications: Obviously when the etiology of a disease remains unknown no effective therapy can be instituted. For suspected metabolic and inherited disorders proper genetic counseling and screening of family members cannot be undertaken. In addition, for patients undergoing transplantation, post transplant follow-up and diagnosis of disease recurrence poses several issues. The patients with CC seem to be slightly older and some studies show a slight preponderance in females and overall a less severe clinical phenotype[4]. The long term outcome and prognosis depends upon the severity of cirrhosis at presentation. Few studies have tried to compare the natural history and prognosis of patients with CC with other common causes of cirrhosis that lack effective treatment like alcoholic cirrhosis and NASH. In one such study the overall survival of CC

was similar to patients with alcoholic cirrhosis[5]. Comparison of patients with CC and NASH undergoing OLT also suggests similar long term outcomes[6,7]. Patients with CC are also at risk for HCC; however, it is thought that the tumor characteristics and outcomes may be different compared to HCC associated with other known disorders. In one such study HCC arising in alcoholic cirrhosis had worse liver function and more aggressive tumor morphology, while the cancers in CC were more often single, less advanced, showed less vascular invasion, more often treatable and had a better median survival[8]. In another study, HCC arising in patients with CC were larger compared to those arising in chronic hepatitis C, but the cancer related mortality was lower[9]. Since disorders contributing to CC likely vary depending on many factors that include historical time period of the study, institutional resources and geographic variation, the results of such comparisons are also likely to show discrepant results, and need to be interpreted carefully.

Suspected etiology of cryptogenic cirrhosis: Histological analysis is an important component in the evaluation of cases that are deemed CC based on clinical work-up only. Many studies showing higher incidence of CC did not take histologic findings into account. In one study of 27 cases of CC that underwent orthotopic liver tranplantation (OLT), histological analysis and follow up led to a more definite diagnosis in 23 of 27 (85%) patients: Nonalcoholic steatohepatitis (NASH) in 9 (33%), autoimmune liver disease (AILD) in 6 (22%), alcoholic liver disease in 4, secondary biliary cirrhosis in 2 and 1 each of hepatitis C and portal venopathy[10]. Other studies show similar findings and suggest that a significant proportion of NASH and AIH cases remain undiagnosed in the absence of histologic examination and contribute to clinically diagnosed CC[6,7,11]. Based on our understanding of the natural history of NASH, its overlapping clinical features with CC and follow up data from OLT literature, it is widely believed that it contributes to about 30% of CC cases. Interestingly, in an era of vastly improved diagnosis of viral hepatitis it has been shown that 20-30% patients with a diagnosis of CC may have occult/cryptic HBV or HCV infection. Other potential causes that remain unrecognized and lead to CC include various inherited and metabolic disorders, non-B/C viral hepatitis and alcohol/drug exposure. The group of inherited and metabolic disorders besides some of the common conditions like hemochromatosis, Wilson's disease, PFICs and AIAT deficiency, also includes many rare disorders such as: Alstrom syndrome (mutations in ALMS1), short telomere syndromes (mutations in *TERT* and hTR) , mitochondrial DNA depletion syndrome (mutations in MPV17) , polymorphisms in glutathione S-transferases, keratin 8 and 18 mutations and Familial Mediterranean fever (mutations in the FMF gene)[12-17] . While the incidence of CC in children and young adults is also similar (about 10%), the etiologic causes are likely different. In our study looking at the causes of cirrhosis in patients <40 years of age showed that metabolic disorders and inherited cholestatic syndromes contribute to a larger proportion of CC cases in children <18 years old as opposed to adults {Gurung, 2015 #189}.

Recent advances in molecular and genomic medicine: Identification of specific mutations for the diagnosis of a disorder has been invaluable for many disorders with

known genetic defects and plays a key role in the diagnosis, especially when the clinical presentation is atypical. However, one can explore only a limited number of genetic mutations either singly or as a "panel" based on the clinical differential diagnosis, even in children where inherited metabolic disorders are common. The limitation of looking at only one gene or a small panel of genes, especially in situations where the clinical presentation is atypical or genetic basis of the disease is unknown is obvious. The enormous cost and technical challenges associated with sequencing the whole genome has restricted its application in clinical practice in the past. However, with next-generation sequencing (NGS) technology the situation has changed dramatically and its impact on many aspects of molecular and cell biology is rapidly becoming very apparent. Genetic alterations are thought to play a major role in Mendelian and non-Mendelian disorders. The overwhelming majority of these disorders are caused by genetic mutations that affect the function of individual proteins, and in the Mendelian disorders, approximately 85% of the disease causing mutations are found in the coding regions of the genome (exome) or in the canonical splice sites. The exome represents only 1% of the entire genome. Thus, while whole-genome sequencing is more comprehensive, whole exome sequencing (WES) is currently more cost-effective. One of the most noticeable outcomes of this new technology has been the accelerated rate of identification of disease-causing genes since the its first successful application in 2009. Since then WES has been shown to be highly sensitive and specific for detection of homozygous and heterozygous variants. As a result, the number of genes previously known to be associated with disease-causing mutations has now been growing at an unprecedented rate within the last few years.

At our institution we have been using WES of germline DNA for the work up of suspected cases of metabolic disorders. In one study of 3 patients with liver disease of unknown etiology we were able to detect mutations in MPV17, SERAC1 and NOTCH2, expanding our knowledge on the spectrum of inherited metabolic liver diseases[17]. In another study using WES in 8 subjects from 6 kindreds with idiopathic portal hypertension, we were able to identify identical rare homozygous p.N46S mutation in *DGUOK*, a deoxyguanosine kinase required for mitochondrial DNA replication[18]. This was a novel and completely unexpected finding. In another patient with hepatic and neurological dysfunction WES revealed a homozygous loss of function mutation in *ACOX2*. This led to the recognition of a yet undescribed disorder of bile acid synthesis that could lead to neurologic disease and cryptogenic cirrhosis. Subsequently in a study of 15 patients with CC we identified unsuspected disease causing mutations in at least 2 patients (data unpublished). The recognition of the underlying disease in such cases has allowed us not only to provide important genetic counseling, but also to understand novel disease mechanisms and provide appropriate treatment. This remains a very exciting area of clinical investigation and one expects that this will eventually become a routine clinical test in any patient with liver disease of unclear etiology.

Patients with neonatal cholestasis present another challenging area in clinical practice. Cases of infantile cholestasis often have very similar clinical presentation despite a diverse etiology[19,20]. After exclusion of common non-genetic causes a huge list of rare differential diagnosis remains to be solved. In recent years the genetic basis of many of these disorders has been elucidated and molecular diagnostic tests are available for a limited gene panels in some of the specialized tertiary care medical centers. However,

more than 90 genes have been associated with monogenic forms of infantile cholestasis, which makes genetic workup by Sanger sequencing time consuming and cost prohibitive. Application of NGS in this setting offers a very cost effective way of not only discovering new disorders, but also identifying novel mutations in some of the known cholestatic diseases. In one recent study of clinically well characterized patients in whom common causes of infantile cholestasis have been excluded six novel mutations (PKHD1: p.Thr777Met, p.Tyr2260Cys;ABCB11: p.Val1112Phe, c.611.1G > A, p.Gly628Trpfs*3 and NPC1:p.Glu391Lys) and two known pathogenic mutations were detected that led to the diagnosis of autosomal recessive polycystic kidney disease, atypical PFIC and Niemanne-Pick syndrome type C1[20] .

Application of NGS in the area of infectious disease diagnosis is another very exciting area that has tremendous potential for the identification of unsuspected hepatic infections. Use of such techniques that are highly sensitive and specific has led to unexpected finding of viral genomes in the evaluation of tissues. For example, application of highly sensitive PCR on liver tissue has demonstrated the presence of HEV, HBV and HCV in CC patients where other routine diagnostic methods from peripheral blood like serology and routine viral RNA/DNA assays had failed[21]. Studies have shown presence of "cryptic" viral infection in 20-50% of CC cases and patients with HCC associated with liver diseases of unknown etiology[22-24]. While this still remains somewhat controversial and an area of evolving knowledge, future research using the technological advances offers a tremendous opportunity for application of these tests in clinical practice.

It is clear that increasing use of these techniques has not only led to the identification of novel disease causing mutations in known genetic disorders, but also to the uncovering of new metabolic disorders. This has led to the expansion of the disease spectrum in some disorders and completely redefined the clinical features in others. The proper interpretation of the data generated from sequencing requires an expert team of geneticists, informatics, pathologists and clinicians. It needs to be said that while NGS technology is now widely available at major medical centers across the world, its clinical application in the identification of genetic disorders and infectious diseases has been somewhat limited by the lack of clinically validated tests and lack of expertise in these areas.

Clinical application and future perspective: It is suspected that besides NASH and AIH, CC group contains many inherited metabolic disorders and cryptic viral infections, and it is anticipated that application of these recent advances will not only lead to the unraveling of genetic and metabolic basis for some of the known disorders but will also help to characterize new disorders. Better understanding of the etiologies and disease mechanisms is likely to result in development of appropriate therapies. Since the technologies and expertise are still available at limited centers and their use in current clinical practice is still somewhat limited. Concurrent advances in the field of metabolomics, proteomics and genomics lend a tremendous opportunity for making significant inroads in this area as well. A combined approach of these technologies is likely to radically change the diagnostic approach in the clinical testing of disorders of unclear etiology, including patients with CC where routine work-up have failed. Once the newer technologies become readily available and widely used, one could predict that the incidence of CC is likely to further decline rapidly. It also implies that the future

definition of CC is likely to include application of molecular and genomic work-up of such cases before considering them truly “cryptogenic”.

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