



# Early Prognostic Predictive System of AECHB and the Diagnosis of Severe Hepatitis B (Liver Failure)

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## Abstract

This chapter describes the evaluations for the progression of chronic liver diseases, recent achievement for screening and early warning-parameters of AECHB, diagnosis criteria and grading standards of HBV ACLF.

1. Several methods have been used to evaluate the progression of chronic liver diseases, including the Child–Turcotte–Pugh (CTP) score, MELD, MESO index, MELD-Na joint formula, iMELD formula, King’s College Hospital (KCH) score, Sequential Organ Failure Assessment (SOFA) score and the Tongji prognostic predictor model (TPPM) score.
2. Technologies used for early screening of severe hepatitis B include gene-based diagnostic techniques, such as the polymerase chain reaction, gene sequence analysis, gene chips, and GWAS. Protein-based methods include two-dimensional gel electrophoresis and mass spectrometry; and epigenetic-based methods include assays of DNA methylation and histone modifications. In addition, metagenomics and systematic biology have been used to analyze microbial sequence and function.
3. Early-warning parameters for severe hepatitis B mainly include serum concentrations of ALT, AST, total bilirubin, albumin and pre-albumin, and cholinesterase; and measurements of blood ammonia, prothrombin time and prothrombin activity. Several new parameters related to severe hepatitis B have been identified, including gene mutations (e.g. HBV 1896 site mutation and 1762/1764

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double mutation), genetic molecular targets (e.g. CXCL10-201 g/A, IL10-592 t/C, ESR1 IVS1-401 t/C, TBX21-1993 t/C, and ICAM1 R241-E469), immune factors (e.g. TNF- $\alpha$ , reactive oxygen species, reactive nitrogen species, sCD163, hfgl2, HLA-DR, NK cells, CTL, Th17 cells, Treg cells, PD-1/PD-L), and metabolic factors (e.g. lecithin, fat amides and bile acids).

4. The clinical diagnosis of severe hepatitis B is mainly based on clinical manifestations, including jaundice, coagulation disorders, hepatic encephalopathy and ascites, and laboratory tests, including prothrombin time, prothrombin activity, international normalized ratio, AST/ALT ratio, and serum concentrations of albumin, bilirubin, cholinesterase, cholesterol, lactic acid, and alpha-fetoprotein.
5. At present, there are differences among countries in the standard for diagnosing liver failure. In China, liver failure is referred to as severe hepatitis, whereas, in western countries, liver failure caused by viruses is diagnosed as fulminant hepatitis and refers only to acute liver failure. The main difference is that, in China, the concept of acute fulminant hepatitis has been extended to patients without encephalopathy. In contrast, hepatic encephalopathy II is a necessary condition to diagnose severe hepatitis in the United States, Europe and Japan.
6. Liver failure can be subdivided into acute and chronic liver failure. Acute liver failure includes acute and sub-acute liver failure, and chronic liver failure includes acute-on-chronic and chronic decompensated liver failure. To date, hepatic encephalopathy has been necessary for the diagnosis of acute, but not chronic, liver failure, which is characterized by decompensated liver.

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### 3.1 Screening for Early Warning Indicators of AECHB

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#### 3.1.1 The Significance of Early Prognostic Prediction for AECHB

Hepatitis B virus (HBV) infection is a serious global health problem and is particularly highly endemic in China. Data obtained from the most recent national HBV survey in China revealed that the prevalence of HBsAg in the general Chinese population was 7.18% in 2006. The total number of HBV carriers is estimated to be more than 93 million, in which 30 million are patients with chronic HBV infection. Chronic hepatitis B (CHB) exhibits a prolonged course and poor prognosis. Liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) are common long-term pathological consequences of CHB. A proportion of HBV carriers and CHB patients can develop into acute, subacute or chronic severe hepatitis in case of rapid deterioration of disease condition. Severe hepatitis caused by HBV infection accounts for

approximately 70% of the total severe hepatitis cases in China. The low treatment efficacy, poor prognosis and high mortality of patients with severe hepatitis cause a serious threat to people's health, greatly increasing the economic burden of families and society, as well as affecting social stability.

To date, antiviral treatment for HBV has achieved remarkable progress. However, the incidence of acute exacerbation of CHB (AECHB) remains high. AECHB is characterized by acute onset, complicated condition, poor prognosis and high mortality. There is a lack of specific and effective warning and treatment for severe hepatitis. Thus, prognostic prediction is challenging and death rate associated with severe hepatitis is high. A comprehensive and accurate understanding of the natural outcome and mechanism of AECHB remains critical. In China, techniques and methods on the prognostic prediction and early diagnosis of severe hepatitis have not been established. The implementation of early intervention of AECHB is limited by the lack of reliable, systematical, sensitive and specific indicators, and a feasible grading system to early predict AECHB and monitor disease progression. As a consequence, some patients may develop to a worse condition and miss the optimal treatment opportunity. At end-stage liver disease or severe hepatitis, treatment efficacy is greatly reduced and death rate significantly increases. Therefore, there is an urgent need to unveil the pathogenesis of AECHB and its clinical manifestations, and establish an early prediction assessment system for AECHB. The establishment of a more specific early detection, intervention, treatment and effective prevention system would increase the cure rate and decrease the morbidity and mortality of AECHB.

### **3.1.2 Techniques and Methods for the Early Prognostic Prediction of AECHB**

A global revolution is booming in the knowledge base of life sciences and biotechnology. This new technological revolution opens up new applications in healthcare, agriculture and food production, environmental protection, as well as new scientific discoveries. With the emergence and development of medical biotechnology, new scientific disciplines such as genomics and bioinformatics appeared and profoundly influenced medical research. At present, the mechanisms of many human diseases can be illustrated at the molecular level, which greatly solicit the exploration of disease treatment strategies. In addition, new techniques offer screening of early warning indicators related to a variety of diseases. The early warning index screening methods applied to AECHB mainly include: diagnosis technology based on gene level such as polymerase chain reaction, gene sequence analysis, gene chip, and genome-wide association studies; techniques based on protein level such as two-dimensional gel electrophoresis and mass spectrometry; epigenetics represented by DNA methylation and histone modification; metagenomics and bioinformatics for the analysis of microbial genome sequence and function.

### 3.1.2.1 Gene Diagnosis Techniques

#### Polymerase Chain Reaction (PCR)

PCR is a technique based on the natural process of DNA replication. PCR *in vitro* synthesizes DNA fragments determined by a pair of synthetic oligonucleotides that specifically bind to the two polynucleotide chains, and this reaction is catalyzed by DNA polymerase. PCR involves three processes: denaturation, annealing and extension. PCR is characterized by its simple operation, time and effort saving, high sensitivity, strong specificity, high productivity, and good repeatability. The discovery of PCR was so revolutionary in genetic technology and is now the most widely used technique in molecular biology research and diagnostics.

For the examination of HBV, the HBV infection state, viral replication level, and prognosis evaluation of the disease can be reflected by accurately quantifying serum HBV DNA using fluorescent quantitative real-time PCR (RT-qPCR). Additionally, with the aid of specific TaqMan probe, it can simultaneously quantify HBV levels, detect HBV genotyping and locus mutation, study the relationship between HBV DNA content and other serum indices, and analyze the correlation between HBV DNA levels and degrees of liver injury.

PCR is not a complicated technique. However, as a sensitive technique for detecting a slight abundance of templates, PCR amplification techniques raise considerable concerns regarding contamination. Experimental contamination would generate false positive results and consequences would be serious in some cases. Therefore, its experimental and operational processes should be conducted in a clean environment. PCR operations should be performed carefully and accurately to avoid any contamination from outside and/or be suitably controlled with an appropriate negative control to get reliable results.

#### Gene Sequence Analysis

Gene sequencing is the process of determining the precise order of nucleotides within a DNA molecule. Due to the rich genetic information contained in DNA sequences, the measurement and analysis of the gene sequence plays a key role in uncovering its genetic nature and encoding. The advantage of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Currently, main gene sequence analyses include: direct sequencing method (DNA double deoxidizing terminal end method, chemical pyrolysis method, pyrophosphate sequencing method, etc.), DNA fingerprint analysis, PCR single-strand conformation polymorphism, PCR sandwich method and the technology of nucleic acid specific structure analysis, etc.

Researchers are now able to identify the HBV virus genotype, mutation and mutation type through the whole base sequencing of specific gene fragments of the HBV genome, as well as the subsequent RT-PCR validation of large amounts of clinical samples. With the screen out of a group of mutation sites that are closely related to the development of AECHB, it generates the possibility to replace the key amino acid of the viral protein to alter the immunogenicity or to affect the replication or transcription of the virus; thus, affecting the development of severe hepatitis.

Further studies to confirm the relationship between these mutations and AECHB, as well as for monitoring these mutations, warrant valuable information for the early warning, prediction and diagnosis of AECHB. These studies would also provide additional treatment strategies and prognosis evaluation of hepatitis B; thus, helping to reduce the morbidity and mortality of AECHB.

### **Gene Chip**

Gene chip is also known as DNA microarray or DNA chips. Gene chip is a kind of DNA sequencing technique that can provide the rapid, parallel and efficient screening and analysis of biological samples. The operation process of gene chip is as follows. First, DNA probes are covalently attached to the surface of a solid support by micro-applicator technique or *in situ* synthesizing technology to generate a 2D DNA probe microarray. Next, it is hybridized with labeled samples, and hybridized signals are detected by a fluorescence scanner and subjected to computer analysis. The working principle of gene chip is similar to that of classical nucleic acid molecule hybridization methods such as northern and southern blots; in which the probe (the known nucleic acid sequence) is hybridized with a complementary target nucleotide sequence in samples, and then qualitative and quantitative analyses are conducted through signal detection. Furthermore, gene chip is featured with high throughput, integration, miniaturization and automation. It is another major technological revolution in life sciences after PCR. Researchers have screened out a number of key molecules and signal pathways involved in severe hepatitis via the analysis of the gene expression profile in a mice model of ConA-induced severe hepatitis by using the gene chip technique and bioinformatics method. These data were further validated on a large number of clinical samples. It is expected that the exploration of gene expression in early severe hepatitis could be used for the early prediction of AECHB and monitoring of the development of this disease.

### **Genome-Wide Association Studies (GWAS)**

GWAS, also known as whole-genome association studies (WGAS), is an examination of many common genetic variants in different individuals to determine if any variant is associated with a trait. GWAS typically focus on associations between single nucleotide polymorphisms (SNPs) and traits such as major diseases. For a certain disease, this method can perform an overall correlation analysis of SNPs on the whole population within the scope of the whole genome, in order to determine the relationship between some SNPs and disease phenotype, and fully reveal genetic markers associated to disease occurrence, development, treatment and prognosis. Recently, three independent large-sample GWAS from the United States, Europe and Japan reported that a racial difference in the rs1297980 site near gene IL-28B exists. These sites were related to the host's ability of HCV spontaneous clearance and response to interferon treatment. Predictably, the application of this method could help determine genetic targets associated with AECHB susceptibility, carry out risk assessments on patients with susceptibility gene, set individualized treatment strategies, and eventually prevent the development of AECHB in hepatitis B patients.

## Metagenome

Metagenome are the sum of all genetic materials in the microbial community, which consists of the genomes of many individual organisms. Humans have evolved intimate symbiotic relationships with a consortium of microbes (microbiome). For example, the gut of humans is verily a microbial ecosystem, whereas the immune system's primary home is in the gut. Hence, gastrointestinal microbiota has a direct effect on the body's immune response, and the composition of symbiotic microbes can reflect human health conditions. Therefore, in addition to the control by the genes of the human itself, the growth, development, immunity, metabolism and disease process of the human body are also regulated by genetic information and flora structure of the symbiotic bacteria.

Metagenomics has emerged as a powerful tool that can be used to analyze microbial communities regardless of the ability of member organisms to be cultured in the laboratory. Metagenomics is based on the genomic analysis of microbial DNA extracted directly from communities in environmental samples. Except intestinal diseases, a number of systemic metabolic diseases and even oncogenesis are related to the microbiota. CHB patients are vulnerable to microecological imbalance, impaired immune barrier function, and declined colonization capacity; which lead to bacterial translocation and endotoxemia, promoting the development of AECHB. In the past few years, Chinese scientists have analyzed the composition of gut microbiota and the metabolic characteristics of patients with CHB and liver cirrhosis. They demonstrated the existence of specific intestinal microflora in liver pathological state and initially proposed that there is intestinal microecological imbalance in patients with AECHB or liver cirrhosis. The proportion of beneficial intestinal bacteria such as bifidobacteria significantly decreases, while the proportion of pathogenic bacteria such as intestinal bacillus, streptococcus and Pasteur bacteria significantly increase. It was revealed that the degree of intestinal microecological imbalance is associated with the severity of liver disease, and correlates with the increase of blood endotoxin level and liver damage. They identified the specific mode of intestinal flora as flora "biomarkers" of liver disease state and progression. The above studies illustrate the possible mechanism of the impact of intestinal flora changes on the intestinal immune barrier function, and consequently on liver disease prognosis. These studies provide theoretical basis for the development of etiologic agents of gut microbiota for the clinical treatment of CHB. Thus, metagenomics study of the intestinal flora plays an important role in the comprehensive management of CHB and prevention of AECHB.

### 3.1.2.2 Protein Diagnostic Techniques

With the completion of the human genome project, proteomic research has become a hot spot in life sciences. Proteins perform a wide variety of activities in cells, including cell shape and inner organization, product manufacture and waste cleanup, and routine maintenance. The regulation of gene transcription, translation, post-translational modification, interaction and the formation of protein complexes

cannot be reflected from the genomics level. Examination of the proteomic, which are responsible for nearly every task of cellular life, would better interpret changes of the cellular microenvironment.

The liver is the body's second largest organ and the largest gland in the human body. The liver plays a major role in metabolism and energy transformation, and has a number of functions in the body. As the most important organ serving glycogen storage, plasma protein synthesis and drug detoxification, the study of liver proteomics is crucial to the understanding of liver disease. HBV mainly infects hepatocytes and interacts with thousands of host genes. HBV pathogenesis is more complicated than the structure of a virus. Proteomics analysis and the establishment of a proteomics database of infected hosts for each period and genotype of CHB offers a new illustration of the pathogenesis of hepatitis, which would provide new ideas and solutions in determining the diagnostic targets of AECHB.

### **Two-Dimensional Gel Electrophoresis**

Two-dimensional Gel Electrophoresis, abbreviated as 2-DE or 2-D electrophoresis, is a form of gel electrophoresis commonly used to analyze proteins. 2-DE is the core technique for proteome research. Mixtures of proteins are separated by two properties in two dimensions on 2D gels. The principle is to utilize the differential charge and molecular weight of proteins. First, proteins are horizontally separated along the pH gradient based on the isoelectric points; and second, proteins would be isolated effectively according to molecular weight along the vertical direction. Currently, the combined use of 2-DE with mass spectrometry can realize the accurate and large-scale identification of proteins.

### **Mass Spectrometry (MS)**

MS is another rapidly developed and commonly used quantitative strategy in proteomic studies. It is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions. MS separates moving ions (charged atoms, molecules or molecular fragments) in the electric field and magnetic field according to their different mass-to-charge ratio, then measures the accurate mass of ions, and finally determines the ionic compounds. A mass spectrum (plural spectra) is a plot of the ion signal as a function of the mass-to-charge ratio.

Serum protein specimens from patients with moderate or AECHB, acute hepatitis B (AHB) in the recovery stage, and HCC associated with hepatitis B have been analyzed by 2-DE. A comprehensive analysis of spot diagrams is expected to determine key proteins in HBV pathogenesis and identify some serological markers that can be used in the early diagnosis of AECHB. There are proteins with obvious changes in serum in the AECHB group, compared with those in other groups. The combined MS analysis identified that  $\alpha$ -antitrypsin expression increased significantly in the AECHB group, suggesting that  $\alpha$ -antitrypsin might be used as a serological marker for developing AECHB, which provides an important implication for the early prediction and diagnosis of CHB exacerbation.

### 3.1.2.3 Epigenetics

The genetic information of the human genome contains two parts: the information stored by the DNA sequence itself and the genetic regulation of gene expression in a certain time and space. Epigenetics is the study of cellular and physiological phenotypic trait variations that are caused by external or environmental factors, which switch genes on and off and affect how cells read genes instead of being caused by changes in the DNA sequence. Epigenetic modifications mainly include DNA methylation, histone modification, RNA related silence, chromatin remodeling and genomic imprinting; among which, DNA methylation modification is the most common epigenetic change.

At present, epigenetics has become a hot spot in the study of hepatitis B. Research have mostly focused on two aspects: the relationship between HBV associated genome-wide hypomethylation or site specificity hypermethylation and the development of hepatitis B associated HCC. However, research on hepatitis B-related epigenetic susceptibility remains limited. In recent years, researchers have qualitatively and quantitatively analyzed genomic methylation status using gene chip technique combined with epigenetics in a high-throughput manner. By comparing methylome from liver tissues with normal and severe hepatitis, they found global methylation loci on the whole genome of hepatitis B patients; and revealed that methylation loci on promoter can regulate the expression of a gene. With functional screening on cell and animal models, several key methylation loci were identified; and were suggested to be closely associated with the severity and prognosis of hepatitis B. Given the advantage of the rich resources of hepatitis B in China, GWAS and genome-wide methylation differential spectrum studies on AECHB are needed in the future to improve the identification of the methylation specific loci and distribution frequency map at different disease states, and to systematically explore the methylation regulating mechanism. These may play a positive role for the prediction, prevention and treatment of AECHB, together with traditional strategies.

### 3.1.2.4 Bioinformatics

The close combinational analyses of gene information and protein structures by computer science, statistics, mathematics and engineering form an interdisciplinary field, bioinformatics. Bioinformatics develops methods and software tools for understanding biological data. It integrates and analyzes large amounts of biological information in experiments with the aid of computer and bioelectronic equipment. Bioinformatics uses mathematical interpretation and the integration of biological experimental data based on information theory and technology to illuminate the significance of biological macromolecules and its implications in health.

Information analyzed by bioinformatics are mainly in three fields: genomic information, protein structure modeling and drug design. These include sequence comparison and analysis, gene identification and function prediction, prediction of protein structure, etc. In China, bioinformatics research has started relatively late, and research on hepatitis B by bioinformatics remains in the early stage. Prediction based on high-throughput chip analysis remains preliminary, which needs further verification. In the future, the combination of molecular biology and



bioinformatics techniques would be the mainstream in the pathogenesis research of major diseases such as hepatitis B. The integration of these techniques would bring hope to the exploration of the early prediction, diagnosis and treatment of AECHB.

### **3.1.2.5 Systems Biology**

Systems biology is a biology-based interdisciplinary field that focuses on complex interactions within biological systems using a holistic approach to biological and biomedical researches. It not only focuses on an individual gene and protein, but the whole biological system. Systems biology integrates the interaction network of cell signal transduction regulation, protein expression regulation, metabolism of tissues and organs, and so on. It explores the occurrence and progression of activities and diseases of the whole biological system from different perspectives by applying bioinformatics and other techniques, and establishing the biological model. Through the dynamic analysis of gene and protein expression characteristics in different courses of hepatitis B, and through its correlation with clinical manifestations, laboratory examinations and overall metabolism conditions, system biology would help screen biological markers for the early diagnosis and predication of AECHB. In the future, systems biology is expected to be a powerful weapon in studying the early diagnosis and prevention of AECHB.

## **3.1.3 Clinical Significance of Early Prognostic Predictive Indicators of AECHB**

### **3.1.3.1 Liver Function and Laboratory Examinations**

The liver is the body's second largest organ and the largest metabolic organ. The liver performs many essential functions related to digestion, metabolism, immunity and the storage of nutrients within the body. These functions make the liver a vital organ, and liver dysfunction can lead to abnormalities in metabolism, synthesis and expression signal transduction molecules, and various chemical substances; and subsequently result in corresponding changes in various serum biochemical parameters. Many early warning indicators have been used in clinical laboratories for liver dysfunction including alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin, prothrombin time (PT) and prothrombin activity (PTA), serum albumin and pre-albumin, serum cholinesterase and blood ammonia. The above indicators also play important roles in the early warning of AECHB. However, none of them are perfect in predicting the severity of liver disease, and a single indicator alone is not enough to accurately predict or diagnose the complicated and rapid changes of liver diseases. In order to accurately determine a condition and make a diagnosis, clinicians must dynamically monitor key serum biochemical parameters and comprehensively analyze clinical manifestations and complications. Thus, to exactly predict the risk of AECHB, it is necessary to weight and score clinical manifestations and laboratory indicators based on established techniques and methods; and to establish hepatitis aggravation-related

mathematical model equations and scoring systems using multiple regression analysis. The establishment of these systems would greatly assist the progress and new breakthroughs in the early warning and diagnosis of AECHB.

### **3.1.3.2 Exploration of Potential Clinical Indicators for Early Warning of AECHB**

Different disease processes and clinical outcomes of AECHB are associated with complicated mechanisms that involves three factors: (1) environmental factors such as the general health and vaccination status of the exposed population; (2) viral factors such as virus genotype, viral mutation, viral load and the number of infected cells; (3) host factors such as age, gender, genetic susceptibility, immune status, the strength of non-specific immune response, complications and other super-infected hepatotropic viruses. Generally, the progress of hepatitis B is a multi-step, multi-gene (host and viral genes) and multifactor (host, viral and environmental factors) interactive process. In order to more comprehensively understand its pathogenesis and explore the potential early warning indicators of AECHB, scientists have conducted a large-scale collaborative research in recent years. They studied the potential mechanisms of AECHB risk from different aspects including viral genomics, regulation of gene transcription, host genetic background, host immunological response and host metabolomics. As a result, they revealed a series of potential early indicators of AECHB to monitor disease progression and severity; which are of great value for establishing reliable, systematic, sensitive and specific early warning indicators of AECHB. These indicators are helpful in formulating a scoring system for the condition of patients, therapy and prognosis; and provide a theoretical basis for reducing the incidence and mortality of hepatitis B.

#### **Viral Factors**

##### **1. HBV Genotype and Subgenotype**

Over the decades, specific genotypes appeared with the accumulation of differences in viral replication. Currently, based on differences in gene sequence between strains, HBV can be divided into A–J (a total ten genotypes); and the A, B, C and F genotypes can be further divided into several subgenotypes. These different genotypes have distinct distributions, and co-infection with virus strains exists in different genotypes. Analysis the PCR amplification products of genome using restriction fragment length polymorphism (RFLP) analysis shows that B and C are the major HBV genotypes in China. Furthermore, the B genotype is distributed mainly in the north, whereas the C genotype is mainly in the south of the Yangtze River. The difference in HBV genotypes affects the transcriptome structure and further viral antigen seroconversion, which results in the diversity of clinical manifestations, antiviral treatment efficacy, hepatitis aggravation and disease outcome in HBV infection. The risk of AECHB is also related to the geographical distinction of HBV genotypes. Among the two major HBV genotypes in Asia, genotype C is vulnerable to hepatitis B aggravation, and severe hepatitis as compared with genotype B. It has been demonstrated that genotype C infected patients were associated with higher serum HBeAg positive rate,

higher HBV DNA level, delayed HBeAg seroconversion, higher transaminase levels, and higher-grade histological inflammatory; indicating that genotype C infection are more prone to severe hepatitis. A multi-center survey in Japan has shown that subgenotype Bj is one of the independent predictors of acute fulminant hepatitis, through a multivariate regression analysis of 301 AHB patients. Another 3-year study on liver failure in the United States reported that the incidence of acute liver failure (ALF) in patients with HBV genotype D was higher than that in patients with the A, B, or C genotypes; suggesting that HBV genotype D may be one of the early warning indicators of AECHB.

## 2. HBV Mutation

As a ubiquitous phenomenon in nature, HBV continuously mutate for survival and adaption to the environment. Due to its high replication activity and lack of proofreading activity in HBV DNA polymerase, HBV exhibits highly genetic variability. HBV viral gene mutations are more common during chronic infection, especially with multi-selection pressure such as antiviral drugs, vaccines, and immune system of the host. The correlation of HBV gene mutation with clinical manifestations, especially with severe hepatitis, is always a hot topic in HBV research. Recently, nucleoside (acid) analogues have been introduced in the management of CHB infection, and have been widely used in clinical practice. Nucleoside analogues reduce the occurrence of severe hepatitis, liver cirrhosis and liver cancer; and improve the life quality of patients. However, they also result in extra iatrogenic variations, besides the genetic mutation of the virus, which made virus variations more complicated; and consequently, more difficult in the early prediction of AECHB and treatment of HBV patients in clinical practice.

Point mutations in the third codon only cause a synonymous mutation that does not change the encoded amino acid and has no biological significance. However, missense mutation may lead to an amino acid change when point mutations occur in the first or second codon position. Key amino acid change in viral epitopes may contribute to virus immune escape and sustained potential infection due to the compromised immune response of the host to the original epitope. In addition, mutations in gene sequences, which are effective drug targets, may result in drug resistance by long-term drug selection of nucleoside (acid) analogues.

Gene mutations can occur on all open reading frames; and sometimes, there may be more than one mutation segment, as revealed by the HBV DNA sequence of the liver tissue. Among these mutations, pre-C/C area variation (pre-C 1896 G-A point mutation and 1762/1764 double mutations in core promoter) are the most frequently reported mutation sites, which is associated with AECHB. Generally, it is believed that HBV pre-C/C genes are the key sites of host immune response and are closely related to the removal of infected and damaged hepatocytes. The pre-C 1896A mutation changes codon 28 encoded amino acid tryptophan (TGG) into a stop codon (TAG), which meant the early termination of HBeAg synthesis and serum HBeAg negative. As a result, the host's immune system finds no targets to attack, leading to "immune escape".

However, sustained replication of the virus may cause CHB patients to easily develop cirrhosis, severe hepatitis and non-response to antiviral treatment. In Japan, the pre-C 1896 termination variation could be detected in 88–100% of patients with ALF and was considered to be one of the independent predictors of fulminant hepatic failure. Core promoter double mutations (C1766T and T1768A) were also reported to be associated with AECHB. These double mutations are located just in the distal nuclear hormone receptor binding sites of promoter C; thus, a similar nuclear factor receptor 3 binding domain exists in the core promoter region. These mutations strengthen HBV transcription and increase HBV DNA load.

HBsAg is involved in hepatotropism, assembly and secretion of viral particles, and induction of specific immune responses. G145R mutation in  $\alpha$  determinants of the gene S region could produce strains that can escape from the hepatitis B vaccine or cause HBsAg negative, while HBV DNA remained positive. This variation appeared in populations inoculated with a variety of hepatitis B vaccines. A chance of being infected by mutant strains and developing varying degrees of hepatitis continues to remain in these vaccinated individuals, even though these individuals harbor anti-HBs antibodies. Consequently, it greatly increases the difficulty of clinical diagnosis and treatment. The C138R mutant in S antigen reduced the level of HBsAg synthesis and the production of neutralizing antibodies by specific T and B lymphocyte. Therefore, this virus cannot be cleared timely, which result in disease aggravation. Simultaneously, variation in the 138 site of HBsAg blocked viral secretion, excessive production and accumulation in hepatocytes that could directly cause liver injury and severe hepatitis.

The HBV P gene encodes reverse transcriptase (RT). Mutations of the P gene affect the catalytic activity of RT and therapeutic target of nucleoside (acid) analogues. The C zone of the RT contains a conserved motif named YMDD (tyrosine-methionine-aspartate-aspartate), which has high enzymatic activity. To date, there are few studies on the relationship between resistance mutations and AECHB. However, there is a natural low proportion of rtV204 or rtI2049 before drug treatment. The rtM204I/V mutation strain can become the dominant strain when the YMDD wild strain is suppressed after L-pyrimidine treatment. Thus, virus sensitivity to nucleoside (acid) analogs is reduced and drug resistance occurs. As a result, the infection with the variant strain persists. A Japanese group reported that rtL180I/V, rtL180M and rtM204I/V mutations were associated with AECHB.

The X gene that encodes the HBx protein is highly conserved. Although the expression of HBx is not high, HBx has complicated biological effects and different targets. The X gene mutation leads to disorders in HBxAg synthesis, and would directly or indirectly affect hepatocyte function, transcription regulation of viral genes, and damage-repair of the host DNA; thus, influencing the prognosis of hepatitis B.

Given that virus mutation is the key factor contributing to the course complexity of severe hepatitis, future studies on the pathogenesis of AECHB are

warranted to further explore the correlation between HBV genotypes or gene mutations and AECHB; which would benefit in determining early warning indicators.

Currently, the reported viral mutation sites were nonspecific in AECHB. In the host, different mutation strains (advantages or disadvantages strains) are mixed together with wild strains in a quasi-species form, which is the long-term mutual interaction between HBV, host, and drug screening. Therefore, to study the relationship between virus mutation and AECHB, the characteristics of HBV quasi-species should be taken into account. By cloning different regions of HBV, Chinese scientists revealed that the complexity and diversity of HBV quasi-species were closely related to AECHB. First, the complexity of the HBV S gene quasi-species in AECHB patients was greater than in HBV carriers and CHB patients. The constitution of quasi-species was more complicated at the 45, 47, 85 amino acids of the HBV S gene in T cell epitopes of CHB patients than HBV carriers. Thus, the difference of HBV S gene quasi-species in T cell epitopes might be related to the clinical outcome of chronic infection. Second, a bioinformatics analysis demonstrated that the complexity and diversity of HBV RT region quasi-species revealed two distinct modes in the response group and non-response group of CHB patients to lamivudine at the 4th week of treatment. This study focused on the dynamic changes of RT district quasi-species and its association with antiviral efficacy in the early stage of nucleotide (acid) analogues-treated CHB patients. The rate of HBV evolution during treatment was significantly higher in the response group as compared with the non-response group, which could be used as a predictor of antiviral effects. Further studies have revealed that the significant reduction of complexity in viral quasi-species in the early treatment with entecavir might be a general rule of effective response to antiviral treatment with nucleotide (acid) analogues. In contrast, there was no significant change on the complexity of quasi-species in non-response or drug-resistant situations. Therefore, the complexity of quasi-species was associated with the severity of HBV disease. Studies on the evolution of a virus *in vivo* and its relationship with hepatitis aggravation would be beneficial to the further exploration of the pathogenic mechanism of AECHB after drug resistance.

### 3. Super-infection

A clinical study found that the incidence of severe hepatitis and the degree of inflammation was higher when CHB patients were super-infected with hepatitis E virus (HEV). A possible explanation is that the liver experiences another acute inflammatory injury by super-infection with HEV in CHB patients, which would cause the degeneration and necrosis of hepatocytes and worsen the condition. Therefore, attention should be given to AECHB in HBV/HEV super-infection.

### 4. Serum Virology

Generally, high levels of serum HBV DNA indicate active virus replication and liver disease activity, and low levels of serum HBV DNA indicate mild symptoms and low infectivity, which require no antiviral therapy. However, in severe hepatitis, host immune response to HBV infection is so strong that HBV DNA is rapidly cleared with the necrosis of a mass of liver cells. As a result, serum levels

of HBV DNA and viral antigens of most patients are low or even negative. Thus, serum viral levels provide minimal information to the early diagnosis of AECHB, and patients should be comprehensively analyzed with other parameters in clinical practice.

## Host Factors

### 1. Age and Gender

The risk to HBV infection is similar between men and women. However, the chance of a chronic, severe progression of hepatitis B is significantly higher in men than in women patients. The younger a person is infected with hepatitis B, the greater the chance of developing chronic infection.

### 2. Genetic Susceptibility

HBV infection leads to a wide spectrum of liver diseases ranging from acute (including fulminant hepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Different individuals infected with HBV have different clinical outcomes. In addition, susceptibility to the same virus, efficacy to the same anti-HBV drug, and immune responses to the same vaccine are also different in distinct populations. These phenomena suggest that there are high variations in host genetic susceptibility to HBV infection.

- (a) Research on the screening and identification of host genetic susceptibility-related genes to AECHB was mainly focused on populations with high incidence in Asia. Using the candidate gene research strategy, recent studies have identified several genes involved in the key immune response signaling pathways in hepatitis B infection such as human leukocyte antigen (HLA), and the promoter region of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-10 (IL-10), and interferon-inducible protein 10 (IP-10; CXCL10).

HLA is the most complex polymorphic gene involved in immune response. The immune genetic basis of HLA can explain the different racial susceptibility to HBV. Therefore, immune responses on gene polymorphism in HLA influence racial susceptibility to HBV, as well as the development and outcome of HBV infection. For example, a genetic association study in Taiwan identified the correlation of HLA-DRB1\* 1001 allele and HLA-DRB1\* 1301, 1302 locus to AECHB and HBV clearance.

In the non-HLA region, there are also HBV susceptibility related genes. It has been shown that the TNF- $\alpha$  gene promoter 308G/A and 238G/A polymorphisms were associated with persistent HBV infection. The frequencies of 1031C, 863A and TNF- $\beta$  B2 allele in the TNF- $\alpha$  promoter region affect TNF- $\alpha$  levels by regulating transcriptional activity; thus, this is significantly associated with the development of AECHB. IL-10 is a potent anti-inflammatory cytokine. Single nucleotide polymorphisms (SNPs) in the IL-10 gene promoter region such as 1082G/A, 819C/T, and 592C/A have also been revealed to be closely related to the progression of HBV infection. Fas ligand (FasL) combined with Fas in infected hepatocytes can induce cell apoptosis. Chinese scientists conducted a binary logistic regression analysis of the relationship between the SNPs of FasL and AECHB after controlling

for age, gender and other risk factors. They found that HBsAg inactive carriers with the FasL-844CC genotype were more vulnerable to severe hepatitis. In addition, chemokines such as IP-10 has also been reported to play an important regulatory role in liver inflammation and injury.

Thus far, results on host genetic susceptibility to hepatitis B were not completely consistent. These controversies may be due to the different sources of research samples or inconsistent viral type in different laboratories. In order to globally and systematically understand the correlation between genetic polymorphisms in HBV infection and AECHB, it is necessary to combine host genetics, viral phenotypes and environmental factors to analyze multiple linked SNPs.

With the completion of the human haplotype map project and the emergence of high-energy SNP genotyping technology in recent years, Chinese scientists launched a GWAS study on AECHB. Through the bioinformatics data analysis of 400 cases of AECHB patients and 400 cases of asymptomatic carriers as controls, they confirmed five AECHB-associated molecular targets: CXCL10-201G/A, IL10-592T/C, ESR1 IVS1-401T/C, TBX21-1993T/C, ICAM1 R241-E469. These loci were analyzed to determine the key locus of genetic variations that affect hepatitis aggravation from a host genome-wide perspective, and clarify the genetic susceptibility to AECHB. In clinical practice, these loci could be used as a risk assessment index of hepatitis B. These loci would also assist in the precision molecular typing, early warning and diagnosis of AECHB; and provide novel and rationale strategies for the prevention and individualized treatment of AECHB.

- (b) In addition to the genetic information stored in the DNA sequence, the human genome also contains a class of genetic information for regulating gene expression levels at a particular time and space, called epigenetics. Epigenetic modifications mainly include DNA methylation, histone modification, RNA related silence, chromatin remodeling and genomic imprinting; among which, DNA methylation modification is the most common in epigenetic changes. Currently, epigenetics has become a hot spot in the study of hepatitis B.

In recent years, Chinese scientists have systematically studied epigenetic modifications during AECHB. Using a high-throughput methylation chip platform, they screened and validated several key sites with methylation changes in the pathogenesis of severe hepatitis. Aberrant methylations of the promoter regions of six genes (IL-17, GSTP1, IFN- $\gamma$ , TNF- $\alpha$ , IL-10, and GSTM3) were shown to be closely related to AECHB. Among these, the methylation/demethylation state of the GSTP1 and GSTM3 promoter region was associated with the prognosis of hepatitis B; which could be used as warning indicators of clinical outcomes. Another important epigenetic modification is RNA-mediated silencing. More attention is now being given on the role of microRNA in immune regulation and clinical outcomes of AECHB. Researchers have performed functional analyses of miRNA targeted genes based on the cluster analysis of miRNA microarray data by using the GO database, and subsequently validated these results using clinical specimens. Scientists have found

that miR-122, miR-1187 and miR-155 play key roles in regulating liver immune response and hepatocyte apoptosis by the specific modulation of the inflammation or apoptosis pathway, respectively. These molecules are implicated to be biomarkers for assessing the degree of hepatocyte damage and therapeutic targets in liver failure.

### 3. Host-immune Factors

The liver is an organ with predominate innate immunity. It is particularly enriched with cells of the innate immune system such as mononuclear-macrophages, NK cells, NKT cells and various T cell subsets. Mononuclear-macrophages (Kupffer cells) and NK cell-mediated immune response occur in the early stage of HBV infection. Liver is also an important site for the differentiation and function execution of specific T cells, and participates in the regulation of local or overall immune response. AECHB is the main process, in which immune response against the virus induces the wide range progressive apoptosis or necrosis of liver cells in a short period. During this process, interactions occur between liver innate immunity and specific immune response, which cause the dysregulation of the liver microenvironment, as well as the apoptosis and necrosis of hepatocytes.

#### (a) Mononuclear-macrophage System

Liver macrophages, also known as Kupffer cells, reside in the gap between endothelial cells in liver blood sinus and account for more than 80% of host tissue macrophages. Kupffer cells play an important role in liver innate immunity, liver bacterial and viral infection, and liver inflammation. Kupffer cells maintain stability in the internal environment. Kupffer cells can engulf and remove microbes from the gut, degraded endotoxins, immune complexes and some toxic drugs. The first-line of anti-HBV response in the liver is the natural immune response, which begins when Kupffer cells are activated by the virus. On one hand, Kupffer cells can devour and remove viruses; and on the other hand, virus-activated Kupffer cells release inflammatory mediators (e.g. chemokines) into the blood stream, which can activate and recruit more inflammatory cells to the liver to participate in liver inflammation.

**Cytokines:** Cytokines released from activated mononuclear-macrophages in severe hepatitis have drawn broad attention. Various inflammatory cytokines or related mediators influence each other and participate in the occurrence and development of AECHB. Some important cytokines or related cell surface molecules are expected to be early prognostic predictive indicators of acute-on-chronic hepatitis B liver failure. TNF- $\alpha$ , mainly produced by activated macrophages, is the effector of macrophage-mediated cytotoxic effects and the critical mediator of severe hepatitis and endotoxin shock. The binding of TNF- $\alpha$  to TNFR can directly cause liver cell apoptosis or necrosis, particularly in those sensitized by HBV and HBV antigens; which increase the possibility of liver failure. TNF- $\alpha$  can activate inflammation-induced NF- $\kappa$ B and MAPK signaling pathways, expand the biological effect of cytokines such as IL-1, IL-6, IL-8 and platelet activating factor (PAF),



enhance the respiratory burst of macrophages, and induce the generation of free radicals; thereby aggravating liver necrosis. Studies have revealed that TNF- $\alpha$  levels were undetectable in HBeAg-negative CHB patients, while TNF- $\alpha$  levels were elevated in most HBeAg-positive CHB patients. Serum and peripheral blood mononuclear cell (PBMC) TNF- $\alpha$  level in severe hepatitis patients is associated with the degree of liver inflammation and necrosis. The higher the TNF- $\alpha$  level, the worse the prognosis of patients became.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS): Macrophages can produce large amounts of ROS and RNS during mitochondrial respiration and enzymatic hydrolysis reactions that have direct cytotoxicity. ROS interacts with cell membranes and generates lipid peroxides and cytotoxic aldehydes. High concentrations of ROS directly induce cell apoptosis or necrosis via cell oxidative stress. In a ConA-induced fulminant hepatitis model, macrophages and ROS released from macrophages are two key pathogenic factors. The exhaustion of macrophages by gadolinium chloride or inhibition of ROS by free radical scavengers may antagonize the occurrence of fulminant hepatitis. Nitric oxide (NO) levels in patients with severe hepatitis also significantly increase, but decline when liver inflammation is relieved and the disease is recovered; suggesting that NO levels can indicate the severity of liver diseases.

Soluble CD163 molecule (sCD163): CD163 is a mononuclear-macrophage population-specific scavenger receptor capable of binding to ox-LDL. CD163 is involved in the identification and removal of pathogens, as well as some aging red blood cells and certain apoptotic cells; and thereby inhibits inflammation. sCD163 levels in patients with ALF has been revealed to significantly increase greater than ten times higher than patients in the control group, indicating that the activation of macrophages participates in the pathogenesis of ALF. sCD163 levels above 26 mg/L suggests a serious condition, poor prognosis, and need for liver transplantation. sCD163 in combination with the King's College Criteria or the MELD scoring system can improve the sensitivity of diagnosing AECHB, and is expected to be a biomarker for evaluating the prognosis of ALF.

Human Fibroleukin-like protein 2 (hfgl2): Hfgl2 is a member of the fibrinogen family and is expressed mainly by activated macrophages and endothelial cells. Prothrombin activity enables hfgl2 to catalyze the conversion of prothrombin to thrombin, initiate the clotting process, and participate in the formation and location of cellulose. It has been demonstrated that patients with AECHB had remarkable elevated expression levels of hfgl2 in Kupffer cells that could initiate the local clotting process and influence the blood supply of a microenvironment through the formation of fibrin deposition and micro-thrombosis in adjacent liver tissues, which would ultimately result in liver cell necrosis. Meanwhile, the expression of hfgl2 in the PBMCs of patients with acute-on-chronic liver failure (ACLF) was significantly enhanced, as well as the level correlated with the severity of liver disease; suggesting that hfgl2 was of significance in predicting the prognosis of CHB.

Human leukocyte antigen (HLA)-DR molecules: HLA-DR is an MHC class II cell surface receptor. The significant decrease of HLA-DR expression in CD14+ peripheral blood monocytes of ALF patients demonstrates the diminished capability of monocytes in initiating the specific immunity and immuno-incompetent state of cells. The reduction of HLA-DR is accompanied with the significant upregulation of serum IL-4, IL-6, IL-10, TNF- $\alpha$  and other inflammatory cytokines. Monitoring the HLA-DR levels can reflect the functional status of peripheral blood monocytes in hepatitis patients, which provide a predictor for the progression and clinical outcomes of the disease.

Endotoxin: Endotoxin, also called lipopolysaccharide (LPS), is a major component of the outer membrane of Gram-negative bacteria. LPS has the ability to induce hepatocyte membrane lipid peroxidation by directly inducing free radical production. In addition, LPS can also activate Kupffer cells to produce large amounts of inflammatory mediators by binding to the TLR4 receptor, causing hepatic microcirculation disorder and further aggravating liver necrosis. In the course of CHB, besides hepatocyte injury, the function of perisinusoidal Kupffer cells diminishes; thus, the scavenging activity of Kupffer cells to LPS weakens. This enhances the chance of intestinal endotoxemia and catabolism *in vivo* to produce excess ammonia, which can result in hepatic encephalopathy. Therefore, endotoxin levels may reflect liver disease progression.

(b) **Natural Killer (NK) Cells**

NK cells are a type of cytotoxic lymphocyte critical to the innate immune system. Small granules in the cytoplasm of NK cells contain special proteins such as perforin and granular enzyme, which play an important role in killing HBV infected cells and secreting anti-viral cytokines. NK cell activity and function are significantly associated with the degree of liver inflammation. Elevated ALT levels are accompanied with the enhanced activity and degranulation effect of NK cells. During the course of liver inflammation, a large number of NK cells can be activated and recruited to the liver. These cells secrete a large quantity of IFN- $\gamma$  to kill hepatocytes through the Fas-FasL, perforin-granular enzyme, NKG2D-NKG2DL pathways. Secreted IFN- $\gamma$  and TNF- $\alpha$  also suppress viral replication. The proportion of NK cells was lower in peripheral blood of AECHB patients than in mild hepatitis patients and healthy people, but the amount of CD56+ cells in the liver was higher. In addition, the killing capability of activated NK cells in the liver was enhanced, which can kill hepatocytes directly and result in liver injury. In contrast, the secretion ability of IFN- $\gamma$  by NK cells in the liver did not exhibit any obvious change; thus, this cannot effectively eliminate viruses. These data suggest that NK cells are one of the important pathogenic mechanisms of AECHB.

(c) **Cytotoxic T Lymphocytes (CTLs)**

CTL-mediated specific cellular immunity plays a key role in cleaning viral infection and is an important factor that causes liver injury by inducing the

apoptosis or necrosis of hepatocytes; which is closely relevant to AECHB. The mechanisms of CTL-mediated immune injury are as follows. Firstly, CTLs secrete cytokines such as IFN- $\gamma$  at the early stage to activate the NF- $\kappa$ B signaling of hepatocytes and remove HBV effectively by the proteasome pathway. This is the non-invasive form of viral clearance. Secondly, during the progression of hepatitis B, HBV-specific antigens stimulate CTLs to become effector cells and release large amounts of granzyme and perforin to kill the target cells. This phase is manifests as cell apoptosis or lysis. Lastly, effector CTLs express FasL to bind to Fas on HBV infected hepatocytes, and further activate the apoptotic cascade. Meanwhile, Fas on CTL membranes can also bind to FasL, causing the apoptosis of CTLs and downregulation of immune response. In severe hepatitis, non-specific immune effects may occur at an earlier stage, but specific CTL responses mainly determine the necrotic degree of hepatocytes; which is associated with the pathogenesis of AECHB. Therefore, the evaluation of the functional state of specific CTLs may reflect the progression of the disease.

(d) **T Helper Cell 17 (Th17)**

Th17, a newly described Th subset that produces IL-17, has great significance in autoimmune diseases and immune defense responses. Th17 cells produce IL-17, IL-17F and IL-22, thereby inducing a massive tissue reaction owing to the broad distribution of IL-17 and IL-22 receptors. Th17-secreted IL-17 can recruit and activate neutrophils, and induce the secretion of chemokine CXCL8. The proliferation and differentiation of Th17 cells require the involvement of IL-6 and transforming growth factor  $\beta$  (TGF- $\beta$ ), in which STAT3-dependent ROR $\gamma$  is a specific transcription factor of Th17. The role of Th17 cells in hepatitis B continuous to attract more attention. Studies have shown that the proportion of Th17 cells in PBMCs from patients with AECHB was significantly higher than in mild hepatitis B patients. In addition, the increase of Th17 cells was closely associated to disease progression and the degree of inflammatory liver injury. Meanwhile, HBcAg escapes immune pressure from Th17 cells via the induction of IL-10 during the process of CHB. The number of Th17 cells in peripheral blood and liver tissues of ACLF patients significantly increased and was closely related to HBV DNA levels, serum ALT/AST and the degree of liver inflammation. In addition, Th17 infiltration to the liver can recruit and activate monocytes and myeloid dendritic cells (mDC) to secrete large amounts of pro-inflammatory cytokines, which further aggregates the pathological injury of the liver. These data suggests that Th17 cells play a key role in AECHB. Thus, in addition to existing detection indices, monitoring Th17 levels can more accurately reflect the level of immune response in patients; which would be of great significance for the early warning and guidance of the clinical management of AECHB.

(e) **Regulatory T (Treg) Cells**

Treg cells are a class of T cell subsets that suppress the immune responses of other cells. The most understood form of Treg cells express CD4, CD25 and

Foxp3, and are known as CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells; in which, FoxP3 is an important transcription factor. Treg cells modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune diseases. Treg cells inhibit the activation and proliferation of T cells mainly through the mode of contact inhibition. Recently, the role of virus-specific immunoregulatory CD4<sup>+</sup>CD25<sup>+</sup> Treg in HBV infection has drawn widespread attention. Treg cells effectively inhibit CTL function via its immunomodulatory effects; thus, HBV cannot be effectively eliminated. A large number of HBV persistently replicate and infect during the chronic process, which has become the hidden peril of AECHB. Scientists have reported that the proportion of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in both PBMCs and liver-infiltrating lymphocytes in AECHB patients was significantly higher than in CHB patients or healthy subjects, and was positively correlated with HBV viral load. CD4<sup>+</sup>CD25<sup>+</sup>Treg cells can also inhibit the proliferation of the same genotype of HBV-specific PBMCs. These results indicate that the level of CD4<sup>+</sup>CD25<sup>+</sup>Treg cell has an important role in the prediction of CHB progression.

(f) **Programmed Death-1 (PD-1)/PD-1 Ligand (PD-L1)**

PD-1/PD-L1 belongs to the CD28 family, which are inhibitory costimulatory molecules mainly expressed in activated T cells, B cells and myeloid cells. PD-1/PD-L1 negatively regulates immune response. Peng et al. reported that PD-1 expression levels in antigen-specific CD8<sup>+</sup> T lymphocytes in PBMCs of CHB patients were significantly higher than in AHB patients and healthy subjects. In addition, there was a positive correlation between the levels of PD-1 and the copy number of HBV DNA. They also revealed that antiviral therapy can decrease PD-1 level in antigen-specific CD8<sup>+</sup> T-cells, and blockade of the PD-1/PD-L1 pathway can significantly promote the proliferation of PBMCs and increase the production of type I interferon in CHB patients.

The overexpression of PD-1/PD-L can specifically inhibit the immune response of T cells and influence the activation effect of activated T cells on dendritic cells. PD-1/PD-L1 overexpression resulted to the low antiviral function of effector T cells, which is associated with persistent viral infection. Long-term stimulation with HBV antigens can upregulate PD-1/PD-L1 expression levels on virus-specific T cells and form an inhibitory signaling pathway to block its strong host immune response, forming a “brake” effect. This kind of negative regulation reduces liver injury. However, it also increases the chance of HBV immune evasion, enhances HBV replication, and weakens the response to inflammation; which in turn easily leads to the development of severe hepatitis. This suggests that this molecule can be used as an early warning indicator to predict the outcome of AECHB.

(g) **T Cell Immunoglobulin and Mucin Domain-containing Molecule 3 (Tim-3)**

Tim-3, another surface molecule with negative regulatory functions, belongs to the T cell immunoglobulin and mucin family. Tim-3 is a cell-surface receptor mainly expressed on mature T cells and CD11b<sup>+</sup> macrophages.

Tim-3 can regulate all T cell subsets. According to an analysis on Tim-3 protein levels in peripheral blood CD4<sup>+</sup> and CD8<sup>+</sup> T cells from hepatitis B patients, it was found that Tim-3 levels in AHB and CHB patients were both higher than in healthy controls; while the proportion of Tim-3<sup>+</sup> T cells was higher in AECHB patients, compared to patients with mild CHB. These studies demonstrate that Tim-3<sup>+</sup> T cell ratios are positively correlated with the severity of liver injury, and serum levels of Tim-3 are closely related to AECHB. When liver inflammation alleviates and acute hepatitis enters into the recovery stage, Tim-3 expression levels in CD4<sup>+</sup> and CD8<sup>+</sup> T cells decrease again. This result suggests the indicator role of Tim-3 for evaluating the severity of hepatitis.

In conclusion, host immune factors play a key role in the process of AECHB. Illustrating host immunological characteristics during the process of AECHB, studying its immunological pathogenesis, identifying early warning indicators of AECHB, and specifically intervening with immunological methods would be beneficial to the monitoring of the disease and effectively delay the severe process; thus, reducing the morbidity and mortality of AECHB.

#### 4. **Host Metabolomics Factors**

The liver is a critical organ for processing metabolites, and plays a vital role in maintaining the metabolism of the body and a relatively stable internal environment. Due to severely impaired liver function, severe hepatitis patients often have systemic metabolic disorders. Thus, biomarkers for predicting the stage and prognosis of severe hepatitis are expected to be determined by the dynamic analysis of metabolite variations.

Currently, China has established a metabolomics platform based on gas chromatography-mass spectrometry (GC/MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC/MS). According to a multivariate data analysis of metabolic profiles of different stages of AECHB, a number of biomarkers such as lysophosphatidylcholine (lyso-PC), fatty acid amide (FAA), and bile acid (BA) were identified as warning indicators for AECHB.

#### 5. **Host Microecological Characteristics**

The composition of a symbiotic microorganism in the body is closely related to the health condition of a person. Gut microbiota imbalance, impaired intestinal barrier function, and decreased intestinal mucosa colonization are present in most CHB patients; and all of these can lead to bacterial translocation and endotoxemia. Meanwhile, the production of intestinal ammonia is increased, while the liver could not effectively transfer blood ammonia into the urea; leading to increased blood ammonia levels. All these changes promote the development of AECHB. Chinese scientists have first reported the specific mode of gut microbiome in patients with AECHB and cirrhosis, according to a study that analyzed gut microbiome composition and its metabolic characteristics in CHB patients with liver cirrhosis. The proportion of beneficial intestinal bacteria such as bifidobacteria significantly decreased, while the proportion of pathogenic bacteria such as intestinal bacillus, streptococcus and Pasteur bacteria significantly

increased. It was revealed that the degree of intestinal microecological imbalance is associated with the severity of liver disease, and correlates with the increase in blood endotoxin levels and liver damage. They identified specific modes of intestinal flora as flora “biomarkers” of liver disease state and progression. These studies suggest that the study of the intestinal flora may have great clinical significance in the comprehensive management of CHB and prevention of AECHB.

### **3.1.4 Problems and Prospects**

In recent years, China has achieved great progress in the study of AECHB. A number of genes associated with AECHB have been cloned, some genetic susceptibility genes associated with immune response have been discovered, and some virus gene mutations associated with AECHB and a number of molecular targets have been found; and these have important prediction significance on disease progression. However, most indicators are obtained from cross-sectional studies. There is a lack of large-scale prospective follow-up studies to evaluate the reliability of these potential indicators in the early warning and diagnosis of AECHB. Due to the sudden onset and complicated disease condition of AECHB, it is definitely difficult for a single indicator to meet the requirement of clinical practice. In the future post-genomic era, with the wide use of proteomics, metabolomics and other techniques, it is necessary to comprehensively consider multiple factors of AECHB patients such as clinical symptoms, laboratory tests, HBV genotype, virus variation, host genetic backgrounds and immune status, to further understand the pathogenesis of AECHB. The analysis of various factors and its corresponding functional performance, using a logistic regression model to exclude the impact of confounding factors, would assist the establishment of an effective early warning scoring system for AECHB. It is also helpful to identify some simple, reliable and rapid diagnostic markers of AECHB that would benefit patients with hepatitis B.

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## **3.2 Early Monitoring Systems of AECHB**

Zhi Chen

### **3.2.1 Current Diagnosis Situation of AECHB**

Hepatic failure is a severe clinical syndrome mainly manifesting as jaundice, coagulation abnormality, hepatic encephalopathy (HE) and ascites caused by massive or submassive necrosis and serious damage to the liver cells, which leads to liver's dysfunction or decompensation of synthesis, detoxification, excretion and bioconversion.

Currently, the diagnosis standard of liver failure is controversial in the world. For example, the name and classification of liver failure as well as the name of clinical diagnostic criteria varies in different countries. In China, liver failure is considered as a result of severe hepatitis, while in western countries, is divided into secondary to paracetamol toxicity and non-paracetamol associated acute liver failure. In Japan, depending on presence of HE, liver failure is divided into severe hepatitis and acute severe hepatitis, which is equivalent to fulminant hepatitis in western countries. The main difference in classification is that in China not all patients without HE are excluded from fulminant hepatitis, while in United States, Europe and Japan HE grades III and IV are considered as poor prognosis markers. Different diagnostic criteria have both advantages and disadvantages. Including patients without HE into severe hepatitis group is helpful for early warning and treatment of liver failure. However, it is better to treat them differently for the significantly different therapeutic effect and prognosis in the two groups. For acute liver failure, Chinese scholars emphasize there is no liver disease history (including past HBV infection), in contrast to European and American scholars who focus on the acute development process. For example, in HBV carriers who have no history of hepatitis or only one sharp attack, the current rapid onset is considered as acute liver failure. Although there are different criteria for the diagnosis of liver failure, it has been gradually tended to consistency. Such as the current classification of liver failure is gradually unified, including acute liver failure (ALF), subacute liver failure (SALF), acute on chronic liver failure (ACLF), and chronic liver failure (CLF). Clinical diagnosis emphasizes the combination of clinical indicators and pathophysiological characteristics. As for HE, it is considered that it is prerequisite for ALF, but not for CLF which is characterized by decompensation.

In October 2006, the first Chinese guidelines on liver failure were published by Infectious Diseases of Chinese Society and of Liver Failure and Artificial Liver association of Chinese Society together. Based on the experience at home and abroad, it reflected the latest progress and consensus on liver failure at that time. According to the progression rate and pathohistological features, liver failure is classified into four types: ALF, SALF, ACLF and CLF. ALF is characterized by sudden onset with grade 2 HE or above within 2 weeks; SALF onsets with grade 2 HE or above within 15 days to 26 weeks; ACLF is characterized by acute decompensation of liver function on the basis of chronic liver disease; CLF is chronic decompensation of liver function caused by gradual impairment of liver function due to liver cirrhosis. In the guideline, ACLF which belonged to the category of chronic severe liver hepatitis before is for the first time introduced into the classification of liver failure, and chronic liver failure was redefined. As a result of these changes these two types of hepatitis are distinguishable. This classification has more similarities to the one used in western countries. ALF and SALF kept the same diagnostic criteria as before; ACLF has a new criterion -de novo appearing liver cell necrosis of variable degrees following the previous chronic liver disease; CLF is mainly characterized by diffuse fibrosis, nodule formation in different sizes and unevenly distributed liver cell necrosis. The definition of ACLF (SALF) used in the guidelines is not completely consistent with European and American

guidelines, as the guideline emphasizes that ACLF is the acute progression based on chronic liver disease including chronic hepatitis and compensated cirrhosis, while in Europe and America ACLF is the occurrence of acute liver failure on the basis of cirrhosis, but acute liver dysfunction on chronic hepatitis or motionless cirrhosis belongs to acute liver failure. According to the relevant recent studies, in 2012, the group of liver failure and artificial liver of infectious diseases branch and the group of severe liver disease and artificial liver study of hepatology branch of Chinese Medical Association updated the hepatic failure diagnosis and treatment guideline, and developed liver failure guideline. The new guidelines were based on the original 2006 edition, while adding the international normalized ratio (INR) into the evaluation of hepatic failure, making it similar to international criteria. At the same time, the new guidelines further refined the diagnosis standard for ACLF: it is a clinical syndrome in which acute or subacute liver decompensation occurs in short time based on chronic liver disease. It manifests as: (1) extreme fatigue and apparent gastrointestinal symptoms; (2) rapid progression of jaundice, serum total bilirubin (TB) is tenfold higher of normal upper limit or raises more than 17.1 mol/L daily; (3) hemorrhage, prolonged prothrombin time (PT)  $\leq 40\%$  (or INR  $\geq 1.5$ ), without other causes; (4) decompensated ascites; (5) with or without HE.

### 3.2.2 Scoring System

Despite constant development of new medications and continuous advances in severe hepatitis therapy, timing and choice of appropriate treatment are still problematic. Therefore it is important to establish a good method of evaluating the patient's condition on the basis of relative clinical information, in order to define the best time for treatment induction or to define the most effective therapy for the patients at the present stage.

In the following text several widely used scoring systems for assessing the severity or prognosis of chronic liver disease are described:

#### 3.2.2.1 Child–Turcotte–Pugh Score

In 1964, Child and Turcotte together proposed Child–Turcotte classification, which included serum albumin and bilirubin, presence of ascites, hepatic encephalopathy (HE) grade and nutritional status. According to these five measures, patients were divided into three grades: A, B and C. But the assessment of nutritional status was depending on the subjective estimate of the attending doctors, and serum albumin and bilirubin, ascites and hepatic encephalopathy would change with treatment, so it is difficult to make a correct judgment. In 1972, Pugh proposed the Child–Turcotte–Pugh (CTP) Score based on the previous Child–Turcotte classification and that is CTP classification we use now. Compared to the Child–Turcotte classification, CTP uses prothrombin time (PT) instead of nutritional status. Each measure is scored from 1 to 3. The sum of the five scores equals the final CTP score. The higher the CTP score is the lower the survival rate is. Now, the CTP classification is used



widely to determine the prognosis of patients with liver disease because of its convenient calculation and data accessibility. However, the CTP classification still has many limitations. Serum albumin levels can be influenced by treatment, which may change a lot in short time. The most important one is that the assessment of ascites and hepatic encephalopathy is not standardized, so the score may vary from one doctor to another.

### 3.2.2.2 Model of End-Stage Liver Disease Scoring System

The Model of End-Stage Liver Disease (MELD) was originally used to predict 3-month survival in patients who had undergone transjugular intrahepatic portosystemic shunt (TIPS) procedure. Later, it was found to be the best predictor of 3 months mortality in hospitalized patients with liver cirrhosis. The formula for the MELD scoring system is  $3.78 \times \log_e(\text{bilirubin [mg/dL]}) + 11.2 \times \log_e(\text{INR}) + 9.57 \times \log_e(\text{creatinine [mg/dL]}) + 6.43$ . The MELD scoring system was chosen as a new basis for prioritizing organ allocation by United Network of Organ Sharing at February 27th, 2002.

Compared to the previous CTP classification, the MELD scoring system has many advantages. First of all, the MELD scoring system excluded subjective index such as ascites and HE grade, so that the condition of patients can be divided accurately. Secondly, the MELD scoring system uses INR instead of PT, avoiding the error of measurement in different laboratories. Finally, the parameters involved in the MELD scoring system are simple, and the only subjective factor was etiology, is more convenient for doctors.

Though the MELD scoring system has been used in many fields, it still has some limitations. The serum creatinine, bilirubin, INR and other measures of MELD scoring system are easily influenced by the extrahepatic factors, which will directly affect the evaluation of liver condition. Furthermore, there are other important factors that are not included which can affect the prognosis such as hyponatremia or renal dysfunction.

### 3.2.2.3 MELD-Na Scoring System

Biggins et al. incorporated serum sodium concentration into MELD, called MELD-Na:  $\text{MELD-Na} = \text{MELD} + 1.59(135 - \text{Na})$ . In their cohort study, they suggested MELD-Na could provide more accurate prediction on survival rate than MELD alone. Samuel et al. used population-wide study on adult candidates for liver transplantation finding that MELD-Na can well predict the survival of patients with liver transplantation.

### 3.2.2.4 MESO Scoring System

Huo et al. analyzed the indexes of 231 cirrhotic patients and introduced a new formula:  $\text{MESO score} = (\text{MELD score} / \text{sNa mEq/L}) \times 10$ . They hypothesized that MESO score would be more accurate as it takes both MELD score and serum sodium into account. However, the majority of patients in this study had HBV infection so the MESO indexes may be more applicable in China than other countries where HCV infection and alcoholism are main causes of hepatitis.

### 3.2.2.5 King's College Hospital Criteria

King's College Hospital (KCH) criteria was proposed by O'Grady et al. in 1989, based on a retrospective study on 588 patients with acute liver failure. According to the causes of acute liver failure, it divided liver failure into acetaminophen induced or non-acetaminophen induced. The latter included viral hepatitis, autoimmune liver disease, hepatolenticular degeneration and other diseases that cause acute liver failure. Details of the KCH criteria are as below (Table 3.1).

The KCH criterion is one of the most widely used prognostic model for ALF and emergency liver transplantation due to ALF in western countries. Simpson et al. analyzed 469 patients in a retrospective cohort study, finding the KCH criterion has a good predictability on the prognosis of ALF. In contrast to that study Yantorno et al. studied 120 cases of ALF caused by various reasons and concluded that MELD was superior to the KCH criteria as a prognostic model. So it may be advised to combine the MELD scoring system and the KCH criteria to evaluate ALF.

### 3.2.2.6 Sequential Organ Failure Assessment

Sequential Organ Failure Assessment (SOFA) was introduced by the European Society of Intensive Care Medicine in 1994. The major cause of death in patients with liver failure was combined with multiple organ dysfunction syndrome (MODS) as there was no effective evaluation method for patients with liver failure combined with MODS before SOFA appeared. Comparing SOFA with the KCH criteria and the MELD scoring system in patients with ALF, Cholongitas et al. found the SOFA performed better than other prognostic models, probably due to its reflection of multiple organ dysfunction.

SOFA score is used to describe the incidence, development and evaluation of the incidence of MODS. As it takes the patient's respiratory, blood, cardiovascular, renal and central nervous systems into account, it is better in estimating the condition of patients with liver failure and MODS. Furthermore, the variables used are continuous and objective, simple, easy to get and reliable. On the other hand, it has some limitations, i.e. it uses only serum bilirubin levels to measure the liver function which is not enough for a complete estimate of it.

**Table 3.1** Meet the KCH criteria suggesting poor prognosis

KCH criteria
<i>Acetaminophen induced</i>
PH of arterial blood <7.30 or meet all the following
(a) PT > 100 (INR > 6.5)
(b) Cr > 300 $\mu\text{mol/L}$
(c) Hepatic encephalopathy at stage 3–4
<i>Non-acetaminophen induced</i>
PT > 100 (INR > 6.5) (no matter the hepatic encephalopathy stage) or meet 3 of the following 5
(a) <10-year-old or >40-year-old
(b) Etiology:
(c) HE occurred more than 7 days after jaundice
(d) INR > 3.5
(e) Serum total bilirubin >17.6 mg/dL

### 3.2.2.7 The Tongji Prognostic Predictor Model

The Tongji prognostic predictor model (TPPM) score was proposed by Ke Ma and Qin Ning et al. in 2013. The TPPM system is a novel model that calculates the TBIL, INR, number of complications and HBV copy number. This model pays close attention to multiorgan disorders and takes the pathogen (HBV) into account.

$$\text{TPPM score } P = 1 / \left( 1 + e^{-\text{logit}(P)} \right)$$

$$\begin{aligned} \text{logit}(P) = & 0.003 \times [\text{TBIL} (\mu\text{mol} / \text{L})] + 0.951 \times \text{INR} + 2.258 \\ & \times \left[ \begin{array}{l} \text{constant for complications : 0 if without or} \\ \text{with 1 complication; 1 with 2 or more complications} \end{array} \right] + 0.114 \\ & \times [\lg \text{HBV DNA} (\text{copies} / \text{mL})] - 5.012 \end{aligned}$$

The TPPM, which takes into account the HBV copy number, was more accurate in predicting outcomes of HBV-ACLF patients compared to the MELD score, which was consistent with the results obtained by Ma et al.

### 3.2.2.8 New Prognostic Evaluation Model

Apart from described above, there are also many other monitoring systems. Many of them are based on MELD scoring system.  $\Delta$ MELD can better reflect the dynamic changes of the disease. iMELD brought age and serum sodium into the scoring system. Shi et al. developed a modified MELD model incorporating age and HE which is better in predicting 3-month mortality in HBV-ACLF patients. According to analysis of 80 patients with fulminant hepatic failure, Miyake et al. proposed a new scoring model, containing cause of fulminant hepatic failure (HBV or indeterminate), hepatic coma grade (III or IV), systemic inflammatory response syndrome and the ratio of total to direct bilirubin.

The key to prevent liver failure is early detection and early treatment. The complicated cause, course, clinical manifestation and complication of liver failure make the early warning, early diagnosis and early prognosis difficult to make. Nowadays, with the development of medical treatment, artificial liver, stem cell transplantation and liver transplantation, the morbidity and mortality decreased, but the overall prognosis is still poor. The most effective way to improve the survival rate of patients with liver failure is to correctly evaluate the prognosis of liver failure and estimate the appropriate time for liver transplantation. As each monitoring system has its advantages and disadvantages, further studies are needed to explore better monitoring system containing various factors influencing the prognosis of liver failure. By using those models we will be able to start an early treatment and improve the prognosis.

## 3.2.3 The Idea of Establishing Early Monitoring System of AECHB

HBV is one of the most widely spread viral infections in China. Infection with HBV has a various clinical presentation ranging from asymptomatic carrier to acute self-limited hepatitis, chronic hepatitis and fulminant hepatitis (FH). Vertical transmission

is the important transmission route of HBV infection. Though patients with chronic infection usually stay in a state of immune tolerance and have no response to HBV, a number of patients deteriorate under certain conditions. As FH usually starts and progresses rapidly, followed by massive or submassive necrosis in short time, there is no specific and effective treatment for it. Most patients have poor prognosis, except in case of early liver transplantation. Admittedly, the interaction of virus and host determines the outcome of AECHB. However, the precise mechanism remains unknown and there is no satisfactory monitoring system. Exploring the pathophysiological mechanisms of AECHB, comprehensively considering the patient's manifestations, laboratory tests, genotypes and variations of HBV, host immune status and genetic background, selecting an early monitoring index, and establishing an effective early monitor system through biostatistics on the weight of these factors, will be helpful in early prevention and treatment, improving efficacy of therapy and reducing morbidity and mortality.

### **1. Monitoring of the clinical progression and laboratory tests of hepatitis B plays key role in early monitoring system of AECHB**

Prior to the typical laboratory results in many patients with severe hepatitis, many clinical symptoms reveal the transformation to the AECHB. Although the indicative meaning of monitoring index is limited, simultaneously monitoring different indexes can remarkably improve the sensitivity and specificity. Endotoxemia occurs when liver fails to inactivate endotoxins which can stimulate phrenic nerve and vagus nerve, leading to fatigue, aggravation, poor appetite, abdominal distention, nausea and vomiting. Rapid progression of jaundice, serum TB raising more than 17.1 mol/L/day or jaundice lasts for a long time, and other symptoms worsening following the jaundice are all suggestive of developing of severe hepatitis. In addition, fetor hepaticus and mild delirium may also be important monitoring factors.

### **2. In-depth research on virology, genetics and immunology of severe hepatitis B lays foundation for early monitoring system for AECHB**

#### **(a) Relationship between virus mutation and AECHB:**

HBV variations are related to the clinical manifestations in patients, and some directly contribute to the occurrence of severe hepatitis. Further studies on mechanisms of HBV mutation and the characteristics of variants in the transcription and biological phenotypes can clarify the pathogenesis of severe hepatitis, and provide clues in finding the early monitoring makers of AECHB development.

The G1895A mutation on PC region imports a termination codon, leading to premature termination of HBeAg translation. Due to the same immunogenicity of HBeAg and HBcAg on T cell, G1895 mutation can alleviate the attack to HBcAg on the surface of infected hepatocytes by cytotoxic T lymphocytes (CTL), leading to immune tolerance. At the same time, HBeAg inadequate synthesis makes immune system attack the infected hepatocytes leading to massive necrosis. A1762T/G1764A mutations on BCP region strengthen the viral replication ability, making the virus spread rapidly

among hepatocytes, resulting in liver failure. Ozasa et al. compared virological characteristics between 40 patients with fulminant hepatitis and 261 patients with acute self-limited hepatitis, finding the G1896A and A1762T/G1764A mutation in 53% and 50% respectively, in patients with fulminant hepatitis, more frequently than in those with acute self-limited hepatitis. Further *in vitro* study confirmed that the replication capacities of Bj subtype of HBV were significantly enhanced based on precore (G1896A) and core-promoter (A1762T/G1764A) mutation. Nagasaki F collected samples from five fulminant hepatitis patients infected with same HBV isolates, and found that G1896A, A1762T and G1764A mutation were present in all of these HBV strains. Based on investigation of 793 HBV patients, it was found that BCP/PC mutation was associated with ACLF and the mortality of ACLF patients with PC mutation was increased.

Apart from the above described two classic mutations, recently several new mutations associated with FH were found, such as at positions 1753, 1766, 1768 in core promoter and 1899, 1862 in precore. Sainokami et al. investigated viral factors in 42 patients with acute HBV infection, finding that mutations in both of the precore (G1896A and/or G1899A) and core promoter (T1753A/C and/or T1745C/G and/or A1762T/G1764A) are associated with FH. Single G1862T mutation can reduce virus replication capability. However, combined with other mutations, it will counteract this inhibition and even enhance the virus replication capability, which may be one of the important reasons for development of FH.

HBcAg is the target antigen for CTL. As a core promoter mutation affects the immune response by causing changes to epitopes and function region in HBcAg, mutation in CTL epitope may be one of the viral factors of AECHB. So far HLA-A2 restricted CTL epitope is of the most in-depth studied. The first and the last two short peptide amino acids are the key sites for HLA-A2 binding, whose mutations may weaken the binding affinity of CTL peptide with HLA-A2, influencing antigen presentation and CTL mediated immune response. Mutations of other amino acids will change the spatial structure of polypeptide, also through affecting the binding of HLA-A2. It is suggested that some changes of HBcAg may enhance the binding of CTL and antigen, and then lead to progression of hepatitis. Recently, Sugiyama et al. found A2339G and G2345A mutations in the function site of proprotein convertase furin, except the well-known G1896A mutation, through analyzing a HBV clone isolated from a FH patient. Further *in vitro* studies showed that A2339G plasmid-transfected HCC cells had increased replication efficiency and accumulation amount of the full-length core protein. The replication efficiency was similar to wild type HBV with furin inhibitor, suggesting that the mutation regulates viral replication by inhibiting the cleavage of furin-like protease on core protein, facilitating the accumulation of HBcAg and HBV replication.

Pre S1/S2 was thought to be the most variable region of HBV, whose deletion mutation occurs most commonly. Changes in nucleotides or amino

acids in this region cause imbalance of large, medium, and small proteins, affecting virion secretion and viral replication. Based on complete genome sequence of HBV obtained from a FH patient and a AH patient with same infection source, Chen et al. found that there is no mutation in precore and core promoter region, while the HBV from FH patients had mutation in S1 (2928, 3067, 3078) suggesting that these mutations may be associated with the onset of FH and that their functions need to be further studied. Research of a neonatal FH caused by mother to infant transmission found mutations in precore (G1896A) and pre-S2 initiation codon with inadequate synthesis of HBeAg and pre-S2. One *in vitro* study found the replication activity of mutant virus was not increased, so it was suggested that FH is associated with the absence of HBeAg and pre-S2, not with viral replication activity. Mutations on S gene can induce the changes of B and T cell epitopes, which may affect its immunogenicity. Khan et al. investigated M133T, G119E and R169P mutations in the S domains and found that the regulation of viral secretion is associated with different mutations. An ntG587A mutation was found in a patient with severe hepatitis, who relapsed after receiving liver transplantation and treatment with specific immune protein. This mutation can cause the 145 amino acid change from glycine to arginine, resulting in gathering of the surface proteins in endoplasmic reticulum. Increased viral replication and viral secretion were found *in vitro*, which may be associated with the progress of AECHB. Recently, new mutations were found in S region, ntT216C, ntG285A. These mutations were correlated with hepatitis exacerbation, and the mutations of these two loci are closely related to the genotype of HBV: mutations in cases with genotype B not C significantly increased with hepatitis exacerbation. This is a new pattern of HBV mutation related to FH, further studies on these mutations may facilitate the early predication of AECHB.

In the follow-up study on a group of patients who progressed from CHB to FH, Ohkawa et al. analyzed the HBV features in the two different stages. Both strains possessed remarkably higher replication activity than wild-type HBV *in vitro*. HBV obtained at chronic stage lacked the ability to synthesize relaxed circular (RC) HBV DNA and secrete RC HBV DNA-containing particle, while HBV obtained at exacerbation stage had this ability. Analysis of viral strains demonstrated a considerable number of mutations on the pre S/S gene at chronic stage exists but not at the end of exacerbation. These mutations had close correlation with the RC HBV DNA synthesis and viral secretion. Furthermore, anergy of strains in chronic stage could be reversed by transfection with the pre S/S protein of wild-type. So they hypothesized the viral strain could regain the ability of synthesizing and secreting RC HBV DNA from the hypermutation state to non-mutation state, which may contributed to the disease exacerbation.

Polymerase gene is the coding gene of reverse transcriptase (RT), closely related to viral replication. As the P and S gene are overlap, P gene mutations are often accompanied with the changes in S protein antigenicity and CTL

epitopes, and S gene mutations can also lead to function changes of RT. The most common mutation in this region is lamivudine (LMV)-resistant mutation, namely YMDD (rtM204V/I) motif mutation. Unreasonable drug withdrawal after LMV-resistance can lead to wild-type HBV mutation, inducing FH rapidly. It was also found that YMDD mutation can reduce the antigenicity of HBsAg. Virological analysis revealed that the HBV quasi species in a FH patient with FCV-resistance and LMV-resistance had various mutations before treatment, while unique mutations occurred after progressing into FH, particularly in the BCP (A1762T, G1764A), polymerase (rtL180M, rtM204V, rtA222T, rtL336V), core (cP5T, cS26A, cV85I, cP135A), surface (sI195M, sM213I), X (xK95Q, xN118T, xK130M, xV131I) proteins. So it is necessary to monitor these mutations in drug-resistant patients with CHB, in order to take precautions against FH.

Surely mutation is one of the most important causes of AECHB, but the relationship between mutation and AECHB needs further investigation. The same mutation of HBV has different outcomes because of the different genotype of HBV, making it difficult to clarify the mechanism of AECHB. We need large-scale screening for patients to obtain mutations and genotypes which are definitely related to AECHB, and to provide basis for the early warning signs of AECHB. Meanwhile, *in vitro* studies are needed to exclude interference factors and to clarify functions of specific mutations.

(b) **AECHB is related to the genetics and immune response of the host:**

Considerable amount of evidence showed the importance of host genetic factors during AECHB. Patients who are diagnosed with CHB for decades have different clinical manifestations with only a small proportion of them progressing into severe hepatitis. The morbidity of severe hepatitis varies significantly among different races (in Asia it was dramatically higher than in Europe or America). The same genotype and HBV gene mutation can be found in asymptomatic carriers, as well as in patients with severe hepatitis. Therefore, we think the genetic features of AECHB are complex and include disease heterogeneity, genetic heterogeneity and different effects of mutations. Currently, studies are mainly focusing on the genetic factors of HBV clearance, cirrhosis and hepatoma caused by HBV infection with just few available regarding of genetic susceptibility to AECHB. Furthermore, the studies on association between AECHB and host genetic factors were almost from Asian. These studies focused on genes involved in immune response pathway of hepatitis B, like TNF- $\alpha$ , TNF- $\beta$ , IL-10, IP-10, VitD receptor (VDR), and HLA. Studies about the correlation between the polymorphism of these genes and clinical stages of hepatitis B found that these genes are close related to AECHB. Wang et al. found that the polymorphisms were mainly located in the promoter region according to the detection of SNPs in IP-10 which is attributed to Th1 pathway. Case-control study showed haplotype increase of 1596T-201A, located in the promoter region of IP-10, increases the risk of CHB. The further function experiments confirmed G-201A as an rSNP and suggested IP-10 it took part in the liver inflammatory and necrotic process of hepatitis B.

AECHB often appeared after the change in host immune state, probably because the chronic immune tolerance was broken and more powerful immune response occurred. When exacerbation occurs, the most prominent immune response is the cascade activation of cytokines, leading to sepsis-like immune system paralysis. Inflammatory factors in liver have positive feedback on the immune response, but too many cytokines will cause damage to the body. In recent years, a number of studies have found that there is a serious imbalance of cytokines expression in FH or fulminant hepatic failure (FHF). IFN- $\lambda$  and TNF- $\alpha$  are two kinds of proinflammatory factors which are massively secreted during severe hepatitis. Stree et al. demonstrated that TNF- $\alpha$  and TNFR (TNF- $\alpha$  receptor) were overexpressed in FHF patients. Ohta et al. found that IFN- $\lambda$  receptor-deficient mice were resistant to HBsAg-specific Th1 cells, proving IFN- $\lambda$  as an essential factor for the pathogenesis of FH. This was further confirmed by expression analysis on intrahepatic cytokines in AECHB patients, which showed that the level of IFN- $\lambda$  in chronic severe hepatitis was significantly higher than those in the CHB and the normal control. IL-10 has an anti-inflammatory effect. The concentration of IL-10 plays a direct role on the balance of immune response. Leifeld et al. reported the imbalance of intrahepatic expression of IL-12, IFN- $\lambda$  and IL-10 in FHB patients. Zou et al. investigated the intrahepatic expression of IFN- $\lambda$ , TNF- $\alpha$  and IL-10 in ACLF patients, finding the intrahepatic expression of proinflammatory factors (IFN- $\lambda$ , TNF- $\alpha$ ) in ACLF was higher than in CHB or in normal control, while anti-inflammatory IL-10 expressions showed no significant difference between these groups. These results suggested that the imbalanced expression of intrahepatic cytokines may contribute to immunopathogenesis in ACLF, and that the incompletely activation of immune network in patients with CHB may lead to ACLF. These findings provided new ideas to the early warning signs and treatment directions of ACLF.

Biochip technique has the ability to detect thousands of transcriptions of the whole genome, and is widely applied in complex biological analysis. A appropriately designed and well implemented biochip experiment can reliably select regulated genes from the tested biological system, helping us to get a global and systemic understanding of the genetic characteristics of complex diseases. Chinese researchers collected liver tissue, blood and urine samples at different stages of hepatitis in mice models and patients, studied the dynamic changes of gene expression, microRNA, proteins and small molecules. By using genomics, proteomics, metabolomics, they identified a series of host factors associated with the pathogenesis of AECHB in the whole genome, gave new ideas to clarify the mechanism of AECHB, and provided new targets for early monitoring and treatment of severe hepatitis B. At the same time, this group took advantage of the large prevalence of viral hepatitis in China, collected up to 2426 samples of HBV carriers, CHB (mild, moderate, severe) patients, and liver failure patients, clinically verified partial targets obtained by before?, finding Tim3, iNOS, MIP2, NGAL



can distinguish those with liver failure from the other patients and can be used to monitor hepatitis B, which is of great significance in guiding clinical treatment.

NK cells, NKT cells, mono-macrophage system, T cells, B cells, endothelial cells and blood coagulation system can all influence the progress and prognosis of AECHB. Using immunohistochemistry, Zou et al. found the number of NK cells in the liver were dramatically higher in FH group than that in normal control, or mild CHB and liver cirrhosis groups, while the number of NK cells in peripheral blood was lower than in other groups, suggesting that NK cells are associated with FH. In FH mice models, NKT cells mediated acute hepatitis and liver damage could be prevented by blocking NKG2D on NKT cells, providing potential target for the treatment of FH. Monocytes and macrophages can produce a large number of cytokines, upregulate HLA-DR and TLR, then stimulate the adaptive immunity. It has been found that the level of TLR2 and TLR4 was positively correlated with the level of serum LPS, which can predict the development of AECHB and can be used as a model for predicting the prognosis of AECHB.

The prevalence of hepatitis B is high in China. It has a great significant to control the occurrence and development of the liver disease, especially to prevent the exacerbation of hepatitis. Further studying of the pathogenesis, establishing a complete early monitoring system, providing the basis for clinical monitoring and control of AECHB are the key points in the research of this major infectious disease. In recent years, China has made great progress in the research of AECHB. First of all, the research teams of AECHB in China have considerable scale and formidable strength, they have laid a solid foundation for the studying of virus infection, variation and replication, the interaction between genetic characteristics and host immune response. In addition, after years of research accumulation, Chinese researchers have established a variety of animal and cell models for hepatitis B, research including severe hepatitis, and have found several HBV mutation sites, host immune response markers and genetic susceptibility genes which are related to AECHB. At the same time, large scale of sample libraries of hepatitis B including AECHB has been established by numbers of clinical and basic laboratories. Making full use of these unique advantages, combining the basic medical sciences to clinical, studying on the interaction between virus and host and their effect on hepatocyte apoptosis and necrosis, Chinese experts have made great progress in the field of liver disease. Currently, our main objective is to find the key target of AECHB based on full understanding of the complexity and periodicity of AECHB in order to prevent the occurrence of AECHB by an early intervention. According to the present data, it is necessary to dynamically evaluate the genotype and gene mutation of HBV, host molecular genetic characteristics and host immune response and once the patients have the high-risk indicators, monitoring or intervention should be undertaken immediately. If the virus titer in hepatitis B patients increases significantly, and mutations associated with exacerbation have been found, doctors should monitor these patients and

undertake an appropriate intervention in a timely manner to prevent its progression, reduce the risk of AECHB and improve the survival rate of these patients. Although the research on AECHB has made gratifying progress, we must recognize that there are still large limitations in the understanding of the AECHB. The understanding of multiple adjustment mechanism of AECHB is limited. Numbers of studies were confined to cellular, molecular and individual level research, however the interaction between each level has not been confirmed. Consequently, the essential factors of AECHB are not well known. In addition, the number of hepatitis B related cellular models and animal models is relatively limited, which also restricted the progress on AECHB research, so it is of top priority to develop new and representative experimental models.

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### **3.3 Diagnosis of AECHB and Severe Hepatitis B (Liver Failure)**

Guang Chen and Qin Ning

Hepatitis B progressed to liver failure is called severe hepatitis B. According to the clinical manifestations and laboratory tests, the evidence of HBV infection which met the diagnostic criteria of severe hepatitis can make the diagnosis of severe hepatitis B.

#### **3.3.1 Diagnosis**

##### **3.3.1.1 Clinical Manifestations**

The principal clinical manifestations of severe hepatitis B include fatigue, severe gastrointestinal symptoms, progressive deepening jaundice, encephalopathy, coagulopathy, ascites, etc., and on the basis of these manifestations would appear complications, such as infection, hepatorenal syndrome, hepatopulmonary syndrome, electrolytes and fluid imbalance, etc. Jaundice, coagulopathy, encephalopathy, and ascites are four key points of the diagnosis of severe hepatitis/liver failure.

Fatigue is most common manifestation of severe hepatitis. The general condition of the patient will worsen. They will be completely bedridden, and they cannot take care of themselves in daily life.

1. Severe gastrointestinal symptoms. The patients showed decreased appetite, upper abdominal discomfort, accompanied by tired of the oil, frequent nausea, vomiting etc. The severity of gastrointestinal symptoms can often reflect the liver lesions; the emergence of intractable hiccup often indicates a poor prognosis.
2. Progressive deepening jaundice. Patients with skin and sclera developed progressive deepening jaundice, manifest as hepatocellular jaundice. Generally

speaking, the higher the level of serum bilirubin in patients shows more heavy damage of liver cells, especially in patients with chronic severe hepatitis. But in the patients with acute severe hepatitis disease, the condition oncoming force, hepatic encephalopathy often appears before jaundice.

3. Hepatic encephalopathy (see Chap. 2, Sect. 2.5).
4. Coagulopathy is one of the most important clinical manifestations of severe hepatitis, the mechanism of which is associated with reduced synthesis of coagulation factors, fibrinolytic system function disorder, platelet count reduction and function defect. The clinical manifestations of coagulopathy can be divided into two categories: spontaneous bleeding and iatrogenic bleeding caused by invasive operation. According to the 1970s literature reports, spontaneous hemorrhage of acute liver failure had a total incidence rate reached as high as 50–70%, in which severely bleeding rate reached more than 30%, but in recent years due to the widespread infusion of fresh frozen plasma, the incidence has decreased significantly. The most common site of bleeding is the upper digestive tract, other parts including the skin injection site, nasopharynx, lung, retroperitoneum, kidney, although intracranial hemorrhage is rare, but often fatal. Acute severe hepatitis patients with digestive tract hemorrhage are mainly caused by gastric mucosal erosion and esophageal, and gastric varices bleeding is common in chronic severe hepatitis patients with cirrhosis.
5. Ascites is the most common sign in severe hepatitis. A small amount of ascites could not be found by physical examination, only through the ultrasonic diagnosis. Moderate ascites shows shifting dullness positive. Patients with more obvious ascites manifest as abdominal distension, shortness of breath and even dare not eat for fear of aggravating abdominal distension. Diagnostic abdominocentesis helps to exclude the existence of secondary infection.
6. Cerebral edema is the most serious complication of severe hepatitis, once occurred, the mortality is as high as 90%, and cerebral hernia would occur in 1/3 patients waiting for liver transplantation [1]. Hypoxia, toxin, abnormal cerebral metabolism and cerebral hemodynamic changes and other factors are major causes of brain edema. The related factors of cerebral edema include grade III/IV hepatic encephalopathy, rapid progressive of hepatic encephalopathy after onset, hyperammonemia, infection, SIRS, needing vasopressin and renal replacement therapy. Patients with liver failure admitted to ICU, where intracerebral pressure (ICP) can be monitored in order to judge the occurring of brain edema [2]. But most hospitals in China, there are no condition to carry out ICP monitor. Wang and Gu proposed in their book “liver failure” [3] that can refer to the following clinical manifestations to judge. The major clinical manifestations: (1) coma deepened rapidly, (2) chemosis, (3) irregular breathing, (4) dyscoria, (5) durative elevator, (6) blood pressure optic disc edema. Minor clinical manifestations: (1) high myodynamia, (2) frequently vomiting. Having the two or more main performances and then referring to the secondary performance, can consider brain edema. Cerebral imaging examination is not an effective method for the diagnosis of cerebral edema in the early stage, but can be used to identify intracranial hemorrhage.

7. Hepatorenal syndrome (see Chap. 2, Sect. 2.3).
8. Hepatopulmonary syndrome (see Chap. 2, Sect. 2.6).
9. Secondary infection. Patients with acute liver failure were found 90% with occur secondary bacterial infections, 32% patients with fungal infection, who almost all with common bacterial infection [4]. The most common infection site is abdomen and lung; secondly infection of urinary tract, biliary tract, skin and soft tissue infection, sepsis can also occur.
10. Fluid, electrolytes and acid-base disturbances. Liver metabolism function in patients with severe hepatitis were seriously damaged, resulting in serious environmental disorder, often prone to fluid, electrolytes and acid-base disturbances. The most common manifestations are hypokalemia and hyponatremia [5].
  - (a) Hypokalemia: Inability to excrete free water resulting from high levels of ADH and aldosterone, eating less, frequent vomiting, using loop diuretics (e.g. furosemide) and thiazide diuretics (e.g. hydrochlorothiazide) commonly cause hypokalemia in severe hepatitis patients. The clinical manifestations are limb muscle weakness, reduced or absent tendon reflexes, abdominal distension, constipation, and even the emergence of paralytic ileus, arrhythmia. Due to more serious intracellular potassium deficiency is more serious, the concentration of serum K<sup>+</sup> can not reflect the situation of potassium deficiency in cells.
  - (b) Hyponatremia in severe hepatitis patients is dilution hyponatremia, mainly caused by antidiuretic hormone inactivated reduction by the liver. Patients intake reduction, vomiting loss, and the use of diuretics can also worsen hyponatremia. The clinical manifestations of hyponatremia often concealed by the symptoms of liver failure itself, but the lack of sodium can cause severe acute low sodium syndrome and low sodium encephalopathy, characterized by disturbance of consciousness, blood pressure drops, etc. Persistent refractory hyponatremia is one of the severe hepatitis patients with end-stage performance.
  - (c) Acid-base imbalance: Because of hypoxemia and high blood ammonia stimulating the respiratory center hyperventilation, respiratory alkalosis, may combine with metabolic acidosis or metabolic alkalosis, is a common type of acid-base imbalance in severe hepatitis patients.

### 3.3.1.2 Laboratory Tests

#### 1. Main biochemical disturbance in severe hepatitis B

Liver is the most important organ in the human body to maintain internal environment homeostasis, its biological functions are very complicated, not only the most important serum protein synthesis including albumin, transporters, clotting factors, hormone and growth factor, but also the main place for various nutrients metabolism, in addition to the secretion and excretion of bile, bioconversion and detoxification function. The liver is the body's largest immune organ. Although as many as thousands of items in biochemical reaction occurs in the

liver, only a few of them can be detected at present in the hospital, many of the test items such as aminotransferase and alkaline phosphatase cannot reflect the liver function, but suggest liver cell damage or bile excretion. Though clinicians cannot accurately evaluate the liver function, the comprehensive evaluation of the laboratory indicators can provide a basis for the diagnosis of severe hepatitis, and help us with any other liver diseases identification, assess the severity of the disease, observe the patient's response to treatment and predict prognosis [6].

- (a) Prothrombin time (PT), prothrombin activity (PTA) and INR (see Chap. 1, Sect. 1.4).
- (b) Albumin (ALB) (see Chap. 1, Sect. 1.4).
- (c) Bilirubin (see Chap. 1, Sect. 1.4).
- (d) AST/ALT ratio (see Chap. 1, Sect. 1.4).
- (e) Cholinesterase (see Chap. 1, Sect. 1.4).
- (f) Cholesterol (see Chap. 1, Sect. 1.4).
- (g) Lactate.
- (h) Pyruvate metabolism produces lactic acid in mitochondria of liver cells. Due to lactic acid metabolism must be under aerobic conditions, so blood lactic acid level can be increased significantly in the condition of liver cells damage. In addition, in liver failure hypoxemia often appears hypoxemia, which can aggravate the lactic acid accumulation. In recent years, lactic acid is widely regarded in the assessment of the importance value of prognosis in patients with acute liver failure. If regarding to blood lactic acid  $>2$  mmol/L as a predictor of poor prognosis in severe hepatitis patients, the sensitivity is 92.3%, specificity is 71.4%, accuracy is 85%.
- (i) AFP

The AFP is the most important serum markers in the diagnosis of primary carcinoma. But for the patients with severe hepatitis, AFP levels predict liver cell regeneration process. This is because in the process of liver regeneration, differentiation of atypical II, III type of hepatic cells can synthesize and secrete AFP. The severe hepatitis patients with high level of AFP have a good prognosis. The change trend of serum AFP has an important value in predicting the prognosis of patients with acute liver failure [7].

### 3.3.2 Current Diagnostic Criteria for Severe Hepatitis B (Liver Failure) in China

#### 1. The revision of "the viral hepatitis prevention and control plan" in 2000

Because of significant differences of etiology in liver failure between China and the America and European countries, the word "severe hepatitis" is Chinese specific name, the diagnosis of hepatitis B induced liver failure is classified as severe hepatitis B. The current diagnostic criteria of severe hepatitis in China amended in 2000 Xi'an conference "Viral Hepatitis Prevention and Control Plan", in which severe hepatitis were divided into acute severe hepatitis, subacute severe hepatitis and chronic severe hepatitis [8]. The diagnostic criteria are as follows:

- (a) Acute severe hepatitis: With the onset of acute jaundice hepatitis, extreme fatigue occurred in 2 weeks, gastrointestinal symptoms, the rapid emergence of second degree or above (according to the grade IV division) hepatic encephalopathy, prothrombin activity less than 40% and exclusion of other causes, diminution and vanish of hepatic dullness, dramatically deepen, jaundice; or jaundice very shallow, not even the presence of jaundice, but with similar symptoms above should consider acute severe hepatitis.
- (b) Subacute severe hepatitis: With the onset of acute icteric hepatitis, appearing extreme fatigue within 15 days to 24 weeks, gastrointestinal symptoms, and significantly prolonged prothrombin time, prothrombin activity less than 40% and exclusion of other causes, jaundice growing rapidly, increase of  $>17.1$  mol/L per day or ten times more than the normal value. The first appear second degree of hepatic encephalopathy, called encephalopathy type (including cerebral edema, cerebral hernia); first appeared ascites and related symptoms (including pleural effusion), known as ascetic severe hepatitis.
- (c) Chronic severe hepatitis: The pathogenesis basis include: Chronic hepatitis or cirrhosis; history of chronic hepatitis B virus carriers; no history of liver disease and HBsAg carriers, but there are signs of chronic liver disease (such as liver palms, spider angioma) changes, images (such as the spleen thickness) and biochemical changes (such as gamma globulin rise high, albumin/globulin ratio decline or inverted); biopsy support chronic hepatitis. Patients with chronic hepatitis B/C, or HBsAg carriers overlap HAV, HEV or other hepatitis virus infection need specific analysis, and should exclude HAV, HEV and other hepatitis virus induced acute or subacute severe hepatitis. The clinical manifestations of chronic severe hepatitis are same with the onset of the sub acute severe hepatitis, and progress to severe hepatitis (prothrombin activity is less than 40%, the serum total bilirubin of ten times greater than normal).

In order to evaluate therapeutic effects and prognosticate the outcome, subacute and chronic severe hepatitis are divided into three stages (early, middle and late stage) according to clinical manifestations. According to the diagnostic criteria, acute and subacute severe hepatitis are roughly corresponding with acute and sub acute liver failure of western criteria, respectively. Acute severe hepatitis B which can be thought of as acute liver failure in patients induced by acute HBV infection with no previous history of liver disease is a part of syndrome of acute liver failure. However, route of transmission of more than 90% of chronic HBV infection patients are mother to child transmission, this kind of patients can be in a relatively long period after the first attack of immune tolerance of hepatitis till developing to severe hepatitis. Chronic hepatitis can develop to severe hepatitis based on the long history of pre C region mutation or overlapping other virus infection etc. This category of patients should be classified as a type of chronic severe hepatitis according to diagnostic criteria of "Guideline for Prevention and Treatment of Viral Hepatitis" in China. However, the pathological

characteristics of the patients is liver cell necrosis newly occurred in the basis of chronic liver disease, its clinical features are on the basis of chronic liver disease and acute liver function decompensation. Obviously this concept of chronic liver failure is not consistent with the Western countries. The diagnosis of “chronic severe hepatitis” is commonly used by doctors in China, but likely cause confusion. Especially in the patients with chronic severe hepatitis is translated as “chronic severe hepatitis” contribution in foreign magazines often misunderstood. An important reason for this is not in line with international standards, which many domestic scholars have been complaining about.

## 2. Guideline for diagnosis and treatment of Liver Failure (revision 2012)

So far, Chinese and foreign scholars did not reach a unified opinion on the definition and classification of liver failure, but for a long time lack of diagnostic and treatment guidelines for liver failure was a major dilemma facing many liver disease doctors, until 2006, Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases and Severe Liver Disease and Artificial Liver Group Chinese Society of Hepatology issued China’s first “ Guideline for Diagnosis and Treatment of Liver Failure”, it makes the problem become history. The important contribution of this guidelines is a clear distinction between the chronic on acute (subacute) concept of liver failure and chronic liver failure, which is with Chinese features and in the same track with international practice. In 2012 it was updated by Liver Failure and Artificial Liver Group, Chinese Society of Infectious Diseases and Severe Liver Disease and Artificial Liver Group, Chinese Society of Hepatology, who It is recommended that the clinical diagnosis of liver failure require a comprehensive analysis on the history, clinical manifestation and auxiliary examination. Liver failure can be divided into four categories: acute liver failure (ALF), subacute liver failure (SALF), acute on chronic liver failure (ACLF) and chronic liver failure (CLF) [9] (criteria of liver failure see Chap. 1, Sect. 1.2).

According to diagnostic criteria of “Guidelines for Liver Failure”, patients of severe hepatitis in China should be classified as acute liver failure, subacute liver failure and acute (or subacute) on chronic liver failure, and chronic liver failure should be roughly corresponding to decompensated cirrhosis. According to the statistics of the clinical features of severe hepatitis B patients in China, more than 70% patients suffered from acute (or subacute) on chronic liver failure, which is mainly due to most chronic HBV infection in China are the mother-to-child transmission patients, who have long history of chronic HBV infections before the occurrence of severe hepatitis B. Only a small number of patients who infected HBV first proceed to be severe hepatitis B. The “greatest contribution” of “Guideline for Liver Failure” in China lies in making the character of liver failure in patients clear, which accounted for the largest proportion, no longer confused with chronic liver failure.

It is important to note that, the definition of acute (or subacute) on chronic liver failure in this guideline is acute hepatic decompensation occurred on the basis of chronic liver disease, but there is no clear definition of “chronic liver

disease foundation". Chronic hepatitis B patients who are common clinically, whose liver function is deteriorating rapidly in a short time on the basis of cirrhosis due to viral load raising rapidly or blow causing by other reason. In order to reflect their characteristics of this kind of patients, clinicians often make a diagnosis of severe chronic hepatitis B and decompensate cirrhosis.

If "chronic liver disease foundation" mentioned in the guidelines is consistent with pathogenesis of chronic severe hepatitis mentioned in "the Viral Hepatitis Prevention and Control Plan (China)", which including chronic hepatitis B virus carrier, chronic hepatitis, liver cirrhosis, so there is no doubt that these patients should be classified as acute on chronic liver failure. But the guidelines did not make clear definition for to "chronic liver disease". Some scholar reported, whether the chronic severe hepatitis happened on the basis of liver cirrhosis, the clinical features and prognosis were different. It is not appropriate to classify severe hepatitis happened on the basis of liver cirrhosis to chronic liver failure.

In accordance with international acknowledgement, the primary difference between chronic liver failure and acute on chronic liver failure is the former one having a natural course of liver cirrhosis, which is a progressive and irreversible process till death or liver transplantation; but the latter one is a sharp deterioration of liver function, which is based on the chronic liver disease (with or without cirrhosis of liver function) triggered by factors out of natural progression, when remove the precipitating factors, the illness is likely to be reversible.

Therefore, under the premises of classifying this kind of patients with decompensated cirrhosis as acute on chronic liver failure, patients with decompensated cirrhosis could be grouped into subtype II according to Kamath classification standard (subtype I is having chronic liver disease with well compensated liver function before liver failure; subtype II is having decompensated liver cirrhosis before liver failure).

### **3.3.3 The Comparison of Diagnostic Criteria for Severe Hepatitis B (Liver Failure) Among East and West**

The common causes of liver failure are drugs, toxin, alcohol, autoimmune disease etc., but not viral hepatitis in America and European countries. So scholar in these countries think of liver failure as a syndrome to study and classify. There is no accepted diagnostic criteria or guidelines for liver failure. As always since the acute liver failure caused by drugs, such as acetaminophen, is most common in America and European countries, classification and diagnostic criteria of acute liver failure has been a focus of concern. The diagnostic criteria for chronic liver failure, which refers to a variety of chronic liver disease slowly progressing to decompensated cirrhosis and end-stage liver disease, is rarely reported. The main characteristic of chronic liver failure patients is an irreversible, and liver transplantation is the only effective treatment. The diagnosis of acute on chronic liver failure is of short history, but in recent years has attracted wide attention in the world especially in the Asia Pacific region. As mentioned, patients of severe hepatitis in China should be



classified as acute liver failure, subacute liver failure and acute (or subacute) on chronic liver failure. In this section, we compare the diagnostic criteria of acute and acute on chronic liver failure between China and abroad.

### 1. Comparison of diagnostic criteria for acute liver failure in foreign countries and China

The earliest review of America and European countries about on the definition and classification of acute liver failure in the history America and European countries, the earliest can be traced back to Lucke and Mallory proposed the fulminant form of epidemic hepatitis in 1946. They distinguished two clinical courses: fulminant, with a rapidly fatal outcome; and a subacute form with a slower course but equally poor prognosis [10].

In 1970, Trey and Davidson [11] in America proposed the item of “fulminant hepatic failure,” defined as “a potentially reversible condition, the consequence of severe liver injury, in which the onset of hepatic encephalopathy was within 8 weeks of the first symptoms of illness, and in the absence of preexisting liver disease”.

In 1986, Gimson [12] in British proposed to replace fulminant hepatic failure in patients with acute liver failure, and defined hepatic encephalopathy and other symptoms of liver failure occurred within 8–24 weeks as late onset hepatic failure (LOHF).

In 1993, O’Grady [13] classified acute liver failure into three categories according to the time interval between appearing jaundice and encephalopathy: hyperacute, acute and subacute three categories according to the time interval between appearing jaundice and encephalopathy. Hyperacute liver failure is the term for cases in which encephalopathy occurs within 7 days of the onset of jaundice; acute liver failure for cases with an interval of between 8 and 28 days from jaundice to encephalopathy; and subacute liver failure is suggested to describe cases with encephalopathy that occurs within 5–12 weeks of the onset of jaundice.

The diagnostic criteria above for acute liver failure are different, but generally the diagnosis of acute liver failure is based on the clinical features of hepatic encephalopathy, the time interval between the onset of symptoms or jaundice and hepatic encephalopathy is the basis of various subtypes. In addition to hepatic encephalopathy, ascites and prothrombin activity are clinical diagnosis points in diagnostic criteria of acute liver failure in China. This is because of due to the higher incidence of acute or subacute liver failure due to hepatitis B virus infection induced acute or subacute liver failure is higher in China than in America and European countries. Such patients are often with severe gastrointestinal symptoms and the onset of jaundice and along with the progression of hepatic encephalopathy may occur. But it is also possible that other serious fatal complications may occur, such as liver and kidney syndrome, hemorrhage, or sepsis, without hepatic encephalopathy. Therefore, the main reasons for the difference between China and America and European countries on the diagnostic criteria for acute liver failure is due to differences in disease distribution.

International Association for the Study of the Liver Subcommittee discussed and formulated recommendation on the nomenclature of acute liver failure and subacute liver failure in India 1996. The experts pointed out that in the diagnostic standard of subacute liver failure in Europe and the United States would not apply to Indian subcontinent and some Asian countries. So the most significant difference of this recommendations and previous classification was is separated separating acute liver failure and subacute liver failure as two independent entities, rather than two subtypes a of clinical comprehensive syndrome. Acute liver failure was a potentially reversible, rapid, sustained progress in liver function decompensation (without the underlying liver disease); the clinical feature is the onset of hepatic encephalopathy within 4 weeks. Acute liver failure can be divided into two subtypes: hyperacute (the onset of hepatic encephalopathy within 10 days) and fulminant (the onset of hepatic encephalopathy within 10–30 days). Subacute liver failure was characterized by progression of liver decompensation with unequivocal evidence of pre-existing liver diseases; the clinical was appearing along with ascites and/or hepatic encephalopathy at the onset of 5–24 weeks.

“The Management of Acute Liver Failure” [14] making published by AASLD in 2005 is widely used as the diagnostic criteria of acute liver failure in America and European countries. The definition of ALF in “The Management of Acute Liver Failure” includes “evidence of coagulation abnormality, usually an INR 1.5, and any degree of mental alteration (encephalopathy) in a patient without preexisting cirrhosis and with an illness of 26 weeks duration. 6 Patients with Wilson disease, vertically-acquired HBV, or autoimmune hepatitis may be included in spite of the possibility of cirrhosis if their disease has only been recognized for 26 weeks.” This diagnostic criterion for acute liver failure criteria is different from the criteria in China. The scope of cover in AASLD criteria is significantly increased; the onset time (less than 26 weeks) is equivalent to time range of acute liver failure and subacute liver failure. But the most significant difference from China’s diagnostic criteria is that the guideline of AASLD 2005 specifically mentioned the group of patients with vertically-acquired HBV who could have acute liver failure, despite the presence of cirrhosis, may also be acute liver failure. They merely focused on the onset of liver disease, but not both onset and the past chronic infection of the past, while in China and other parts of Asia, which this group of patients are diagnosed with should belong to acute- on-chronic liver failure.

## 2. Comparisons of ACLF diagnostic criteria between China and other international organizations

In 2002, Jalan and Williams defined ACLF as “an acute deterioration in liver function in a patient with previously well-compensated chronic liver disease due to the effects of a precipitating event such as sepsis or upper gastrointestinal (UGI) bleeding. Acute deterioration in live function over a period of 2–4 weeks leading to severe deterioration in clinical status with a high Sequential Organ Failure Assessment (SOFA)/Acute Physiology and Chronic Health Evaluation II

(APACHE II) score with jaundice and either hepatic encephalopathy or renal failure” [15]. They considered two pathways which may lead to acute deterioration in liver function of patients with previously stable liver disease. “First, the acute component of the liver injury may be due to the effects of a known hepatotoxic factor such as superimposed viral infection with a hepatotropic virus, drug reaction, ingestion of a hepatotoxin or excessive alcohol consumption. Second, the liver injury may be the result of precipitating factors such as variceal bleeding and sepsis which, though not necessarily acting as specific hepatotoxins, have important secondary end-organ-damaging effects on the liver” [15]. The definition of acute on chronic liver failure promoted by Jalan and Williams, which focus on multi-organ failure induced by liver failure, have important value for China’s ACLF diagnostic criteria.

Asian Pacific Association for the study of the liver (APASL) first published an ACLF consensus recommendations in academia. Definition of ACLF (APASL 2008) is “Acute hepatic insult manifesting as jaundice (serum bilirubin  $\geq 5$  mg/dL (85  $\mu\text{mol/L}$ ) and coagulopathy (INR  $\geq 1.5$  or prothrombin activity  $< 40\%$ ), complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease” [16]. Defining the underlying chronic liver disease in ACLF include compensatory cirrhosis of various causes, chronic hepatitis, non-alcoholic liver diseases, Wilson’s disease, metabolic liver disease, but not include steatosis. The acute events in ACLF include infectious etiology, noninfectious etiology and unknown hepatotoxic etiology. The infectious etiology include hepatotropic and non-hepatotropic viruses, reactivation of hepatitis B (overt or occult) or hepatitis C, and other infectious agents afflicting the liver; The noninfectious etiology include alcohol (active drinking within the last), use of hepatotoxic drugs or herbs, flare of autoimmune hepatitis or Wilson’s disease, surgical intervention and variceal bleeding. An analysis of the AARC data revealed that patients with a bilirubin between 5 and 10 mg/dL also had substantial mortality ranging around 38%. The original value of 5 mg/dL was accepted as the cut off for bilirubin for defining liver failure.

Due to the rapid advancements in the knowledge and available information, a consortium of members from countries across Asia Pacific, “APASL ACLF Research Consortium (AARC),” was formed in 2012 [17]. Based on the AARC data, new ACLF consensus was proposed in 2014. In this Vision, liver failure grading, and its impact on the “Golden Therapeutic Window,” extra-hepatic organ failure and development of sepsis were analyzed. New management options including the algorithms for the management of coagulation disorders, renal replacement therapy, sepsis, variceal bleed, antivirals, and criteria for liver transplantation for ACLF patients were proposed [17]. Comparing with consensus in 2009, this new version has recommended that the liver failure should be classified into different grades to predict the outcome, though it still needs further prospective evaluation. The inclusion criteria of extra-hepatic organ failure were raised. In particular, early identification of cerebral failure and renal failure were proposed owing to the close relationship between multi-organ fail-

ure and high mortality in ACLF. Updated information on prognostic scoring systems is included. Lastly treatment recommendations are more emphasized towards urgent intervention during “golden window” [18].

The consensus statement made by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver (AASLD–EASL) mainly emphasizes predisposed cirrhosis and single or multiple organ failures, whereas that by the Asian Pacific Association for the Study of the Liver (APASL) focuses on acute deterioration of previous chronic liver diseases such as chronic hepatitis and/or cirrhosis.

The comparison between the three definitions of ACLF are summarized in Table 3.2.

**Table 3.2** The comparison between the three definitions of ACLF

	China [9, 19]	APASL [17]	AASLD/ESAL [20]
Chronic liver disease	Hepatitis (ESP HBV)	Hepatitis and cirrhosis	Liver cirrhosis
Most common precipitating event	Hepatitis (ESP HBV)	Hepatitis (ESP HBV)	Alcoholic hepatitis
Latent period	4 weeks	4 weeks	Not mentioned
Definition/diagnostic criteria	(a) Fatigue with gastrointestinal tract symptoms; (b) rapidly deepening jaundice, with TBil ten times greater than the ULN, or a daily increase $\geq 17.1 \mu\text{mol/L}$ ; (c) hemorrhagic tendency with INR $\geq 1.5$ or PTA $\leq 40\%$ and other causes have been excluded; (d) progressive reduction in liver size; and (e) occurrence of HE	(a) Jaundice (serum bilirubin $\geq 5 \text{ mg/dL}$ ( $85 \mu\text{mol/L}$ ); (b) coagulopathy (INR $\geq 1.5$ or prothrombin activity $\leq 40\%$ ); (c) complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/cirrhosis	An acute deterioration of pre-existing chronic liver disease, usually related to a precipitating event and associated with increased mortality at 3 months due to multisystem organ failure
Prognostic models	Not mentioned	APACHE II/III, SAPS II, OSF/SOFA	CLIF-SOFA
Organ failure	Focus on liver failure	Focus on liver failure	Focus on multi-organ failure
SIRS/sepsis	Not mentioned	Whether sepsis/SIRS is a consequence of or a cause of liver failure is not clear from the current data on ACLF	SIRS worsen systemic and hepatic hemodynamics/liver function

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