1 Clinical Manifestations and Laboratory Tests of AECHB and Severe Hepatitis (Liver Failure)

Liang Peng, Zhi-Liang Gao, Yu-Ming Wang, Deng-Ming He, Jing-Ming Zhao, Xue-Fan Bai, and Xiao-Jing Wang

Abstract

This chapter describes the clinical symptoms and signs of AECHB and HBV ACLF, classification, grading of HBV ACLF and their features, diagnostic principles and standards in liver pathology, biochemistry, and virology of HBV ACLF.

1. Liver failure is defined as serious damage to the liver cause by a variety of etiologies, leading to liver function disorder or even decompensation, and clinical syndromes with coagulopathy, jaundice, hepatic encephalopathy, and ascites.
2. Severe hepatitis B can be indicated pathologically by apparent hepatocellular necrosis, including extensive multifocal, confluent, bridging, sub-massive or massive necrosis.

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L. Peng · Z.-L. Gao (✉)
The Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
e-mail: pliang@mail.sysu.edu.cn

Y.-M. Wang · D.-M. He
Southwest Hospital, The First Hospital Affiliated To AMU, Chongqing, Sichuan, China

J.-M. Zhao
Beijing 302 Hospital, Beijing, China

X.-F. Bai
Tangdu Hospital, The Fourth Military Medical University, Shanxi, China

X.-J. Wang
Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

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3. Laboratory tests during the course of severe exacerbation of chronic hepatitis B can reflect pathological changes and liver function in a timely manner, providing objective and informative reference data for evaluation of disease severity and treatment efficacy. Among the most important laboratory tests are those for prothrombin activity, international normalized ratio, and increases in total bilirubin concentration.
4. Severe hepatitis B is associated with interactions between the virus and host factors. Detection of HBV DNA, HBV genotype, quasispecies and HBV mutation can provide important theoretical bases for the prevention, control or mitigation of the progress of severe hepatitis B.
5. Noninvasive imaging modalities can be used to visualize the entire liver and parts of it. Measuring liver volume to evaluate liver size and liver reserve capacity is regarded as important in diagnosis, surgical approach and prognostic evaluation of patients with severe exacerbation of chronic hepatitis B and liver failure.
6. Model for End-Stage Liver Disease (MELD) is the first quantitative method developed to assess whether a patient with liver failure requires a liver transplant. The predictive value of the MELD model has been improved by the MELD-Na, iMELD, and MESO models. Several other valuable prognostic models have been developed. For example, for patients with HBV-ACLF, the established TPPM scoring system was found to be more predictive than MELD score.

1.1 Clinical Manifestations of Hepatitis B Aggravation and Severe Hepatitis (Liver Failure)

Liang Peng, ZL Huang, YY Mei and Zhi-Liang Gao

1.1.1 Definitions and Clinical Classifications of Severe Hepatitis and Liver Failure

Currently, both clinical and pathophysiological diagnoses are made of severe hepatitis (liver failure) in China. According to the Guideline for the Prevention and Treatment of Viral Hepatitis (2000), severe hepatitis is classified as acute severe hepatitis, subacute severe hepatitis, and chronic severe hepatitis.

Acute severe hepatitis is initially diagnosed due to acute jaundice that rapidly progresses to liver failure within 2 weeks. Subacute severe hepatitis can be identified in patients with acute jaundice hepatitis that progresses to liver failure anywhere from 15 days to 24 weeks. Chronic severe hepatitis often develops with pre-existing chronic liver diseases. The clinical manifestations of chronic severe hepatitis are similar to those of subacute severe hepatitis in some patients, or, in some patients, appear similar to decompensated cirrhosis at disease onset. The diagnostic criteria for severe hepatitis in China remain to be fully developed and hence have not been introduced internationally.

To meet the clinical requirements and standardize the diagnosis and therapy of liver failure, the Branch of Infectious Diseases and the Branch of Hepatology of the Chinese Medical Association invited experts in China to develop the first Guidelines for the Diagnosis and Therapy of Liver Failure in 2006. In those Guidelines, liver failure refers to severe liver damage caused by multiple factors. That damage to the liver results in either the severe impairment or decompensation of synthesis, detoxication, excretion, and biotransformation in the liver and subsequent clinical manifestations characterized by coagulation disorder, jaundice, hepatic encephalopathy, and ascites. On the basis of pathological features and disease progression, liver failure is classified as acute liver failure (ALF), subacute liver failure (SALF), acute-on-chronic liver failure (ACLF), and chronic liver failure (CLF).

ALF is characterized by the rapid appearance of clinical manifestations. Patients with ALF usually develop a clinical syndrome of liver failure characterized by high-grade hepatic encephalopathy (HE, >grade 2) within 2 weeks. Patients with SALF typically present with a clinical syndrome of liver failure anywhere from 15 days to 26 weeks. Finally, ACLF refers to the acute decompensation of the liver function in the presence of pre-existing chronic liver diseases, and CLF refers to chronic decompensation of the liver function characterized by ascites or portal hypertension, coagulation disorder, and HE due to progressive liver dysfunction in the presence of hepatic cirrhosis. The published Guidelines systemically and extensively reflect the current status of the diagnosis and therapy of liver failure. In addition, the Guidelines, for the first time, focus on liver failure rather than severe hepatitis, which broadens our horizons and highlights practicability.

In China, acute severe hepatitis, subacute severe hepatitis, and chronic severe hepatitis correspond closely to ALF, SLF, and ACLF, respectively, as illustrated in Table 1.1. In some patients, chronic severe hepatitis is similar to CLF in other

Table 1.1 Description and comparison of liver failure and severe hepatitis

Types of liver failure	Definition	Corresponding severe hepatitis
Acute liver failure	Abrupt onset of disease, development of liver failure characterized by hepatic encephalopathy of >grade 2 within 2 weeks	Chronic severe hepatitis with acute onset in patients with acute severe hepatitis, HBV carriers, and chronic hepatitis B patients with mild liver lesions
Subacute liver failure	Abrupt onset of disease and development of clinical manifestations of liver failure between 15 days and 26 weeks	Subacute onset of chronic severe hepatitis in subacute severe hepatitis patients, HBV carriers, and chronic hepatitis B patients with mild liver lesions
Acute-on-chronic liver failure	Acute decompensated liver function in the presence of chronic liver disease	Chronic severe hepatitis in the presence of chronic liver disease (characterized by chronic hepatitis and compensated hepatic cirrhosis)
Chronic liver failure	Chronic decompensated liver function in the presence of hepatic cirrhosis	Decompensated hepatic cirrhosis

HBV hepatitis B virus

Table 1.2 Laboratory test index of AECHB

Index	Mild CHB	Moderate CHB	Severe CHB	ACLF
ALT or AST	≤3 × ULN	>3 × ULN	>3 × ULN	>3 × ULN
TBil	≤2 × ULN	(2~5) × ULN	>5 × ULN	>10 × ULN or increase >1 mg/dL daily
PTA (%)	>70	70–60	60–40	<40

countries. On the basis of available Guidelines for liver failure, we define severe hepatitis B as liver failure due to hepatitis B virus infection. CLF is the most common, and ALF and SLF are rare. Acute exacerbation of chronic hepatitis B (AECHB) is a dynamic process, including mild, moderate, severe chronic hepatitis B and chronic ACLF defined in above guidelines. The reference index of abnormality in laboratory examination is shown in Table 1.2.

In addition to viral replication, other factors also contribute to the pathogenesis of hepatitis B-induced liver failure, such as concomitant infection of other hepatitis viruses (especially the hepatitis E virus), immune status, pregnancy, drug and/or alcohol consumption, concomitant bacterial infection, mental stress, and concomitant disease processes (e.g., hyperthyroidism).

1.1.2 Clinical Manifestations and Complications

The liver is the largest solid organ in humans and has complex functions. Hepatic parenchymal cells are responsible for metabolism, secretion, synthesis and bioconversion. Factors that can cause severe damage to hepatocytes (i.e., parenchymal cells, Kupffer cells) may result in disorders of metabolism, secretion, synthesis, detoxication and immunity. In turn, that damage can lead to jaundice, liver shrinkage, coagulation dysfunction, hemorrhage, secondary infection, hepatorenal syndrome, HE, and other clinical entities described in detail here.

1.1.2.1 Common Clinical Manifestations

General Condition

The physical condition of patients deteriorates, and affected individuals usually develop weakness, extreme fatigue, and a severely diminished quality of life. They frequently require assistance to perform basic personal needs, such washing their face, brushing their teeth, and using the toilet.

Gastrointestinal Manifestations

In the early stage of jaundice, in addition to developing extreme fatigue, gastrointestinal symptoms become evident, including extremely poor appetite, anorexia, intolerance of oily foods, nausea, vomiting, abdominal discomfort, and hiccups. In the jaundice stage, the gastrointestinal symptoms deteriorate further. Patients can develop refractory vomiting, hiccups, evident abdominal distension, and reduced/lack of borborygmus.

Jaundice

Clinically, patients initially note their urine color darkens, becoming a strong-tea like color. Next, a yellowish pigmentation of the ski and conjunctival membranes develops. That jaundice progressively becomes deeper, characterized by hepatocellular jaundice. In this stage, serum bilirubin increases rapidly. In fact, the daily increment in serum bilirubin may be >17 μmol/L (>1 mg/dL).

Hepatic Foetor

The sulfur-containing amino acids in the intestine are degraded into mercaptans that have the odor of rotting fruit. Mercaptans cannot be metabolized in the liver and are therefore excreted from the respiratory tract. This distinctive odor is specifically noted in patients with HE. The severity of hepatic foetor may, in some cases, reflect the severity of liver injury.

Coagulation Dysfunction

The occurrence of coagulation dysfunction is primarily ascribed to the reduced synthesis of coagulation factors by the liver. A majority of the both coagulation and anticoagulant factors are synthesized in the liver. In addition, some coagulation-related factors and their inhibitors are also metabolized in the liver. The outcome of coagulation dysfunction is dependent on the severity of damage to the hepatocytes. Thus, even patients in an early stage of liver failure may present with coagulation dysfunction. Prothrombin (PT) activity is often abnormal in the early stages of liver failure and may therefore serve as a sensitive indicator for the prognosis of liver failure.

Common clinical manifestations of coagulation dysfunction are mucocutaneous bleeding (i.e., spontaneous bruising, gingival bleeding, subconjunctival hemorrhage), ecchymosis at the site of injection/puncture, and purpura in more severe cases. Gastrointestinal bleeding is also common in affected individuals, whereas bleeding into/from the genitourinary tract, lung, kidney, and retroperitoneum is rare but occasionally observed in some patients. If intracranial hemorrhage develops, it is frequently life threatening. In AHF, the incidence of bleeding and severe bleeding is as high as 73 and >30%, respectively. Another cause of coagulation dysfunction is thrombocytopenia and platelet dysfunction. Of the two, thrombocytopenia is more common. Because platelets are derived from megakaryocytes in bone marrow, bone marrow fibrosis and either reduced bone marrow regeneration or invasion of lymphoma cells in the bone marrow can reduce the number of platelets. Platelets perform multiple activities, including adhesion, aggregation, release, and shrinking blood clots. Additionally, they play an important role in coagulation. Platelet dysfunction may also increase capillary permeability and fragility, which may cause either spontaneous bleeding of the skin and mucous membranes or difficult hemostasis following vascular injury.

In patients with SLF, thrombocytopenia is mainly diagnosed in the latter stage of disease in which massive hepatocyte necrosis leads to posthepatic cirrhosis, portal hypertension, and hypersplenism. In CLF patients, thrombocytopenia might be present, and hepatocyte necrosis may aggravate portal hypertension and

hypersplenism, resulting in worsening thrombocytopenia. Splenomegaly and splenic sinus hyperplasia increase the phagocytosis and destruction of platelets. Further, splenomegaly can cause enlargement of the platelet pool within the spleen. As a result, the platelets in the spleen may account for >90% of platelets in the body. The above pathological changes may finally cause a reduction in the circulating platelets. The reason for thrombocytopenia in liver disease patients without hypersplenism is still poorly understood and might be ascribed to following factors (1) the hepatitis B virus may significantly inhibit the megakaryocyte system of the bone marrow, resulting in reduced production of platelets; (2) the thrombopoietin (TPO) level is reduced. The division of megakaryocytes into platelets in the bone marrow is controlled by both megakaryocyte colony stimulating factor (Meg-CSF) and TPO. Meg-CSF primarily regulates the proliferation of megakaryocyte progenitor cells, whereas TPO stimulates the maturation of megakaryocytes and production of platelets. TPO is almost exclusively produced by hepatocytes, and only a minority of TPO is produced in the kidney and other organs. TPO is a key factor affecting the production of platelets, and the synthesis of TPO is reduced significantly in patients with either severe hepatitis or hepatic cirrhosis, which affects the production of platelets. In patients with parenchymal liver diseases, abnormalities of platelets are present in both quality and quantity. For example, when the platelet membrane glycoprotein GPI6-IX is reduced, the aggregation of platelets following ristocetin treatment and the shrinkage of blood clots are markedly compromised; and (3) patients with liver diseases usually develop immune dysfunction and are therefore susceptible to infection. Bacterial toxins and systemic inflammatory response syndrome may also cause thrombocytopenia. One published study of ICU patients found that infection was an independent risk factor of thrombocytopenia.

1.1.2.2 Complications of Liver Failure

HE

HE is both a neuropsychiatric syndrome, a type of central nervous system dysfunction, and metabolic disturbance due to hepatocellular dysfunction and portosystemic shunting. HE is clinically characterized by mental and neurological abnormalities, such as abnormal personality and behaviors, irritability, sleep perversion, drowsiness, and complete loss of consciousness or coma. HE is one of the major causes of severe complications and death in patients with liver failure and is typically classified into one of the four following stages:

Stage 1: the prodromal stage. This stage usually manifests with mild abnormal personality changes and behaviors, such as euphoric excitement, indifference, taciturnity, being sloppily dressed, and inappropriate defecation/urination. The affected individual can usually provide correct responses to questions but they are inarticulate and have slow speech. Flapping tremor/hepatic tremor might also be present. To test for flapping tremor, patients are asked to close their eyes with their arms stretching straight, elbows flexed, palms in dorsal extension, with separated fingers. A positive response is determined when the metacarpophalangeal joint, wrist, elbow, and shoulder show irregular movements (jitter) when held in that position within

30 s. Physicians may also ask the patients to hold the their hand for 1 min. If the physician feels the hand tremor, the test suggests a positive diagnosis of flapping tremor. The condition is caused by afferent dysfunction of joint-reticular formation of the brainstem and a characteristic neurological manifestation. That said, flapping tremor has no specificity fro HE and can also be found in patients with either uremia or hypoxemia due to chronic respiratory disease/heart failure. The presence of flapping tremor in a patient with severe liver disease, however, is helpful for early diagnosis of HE. Patients with HE usually have a normal electroencephalogram. Stage 1 of HE lasts anywhere from several days to several weeks. Several patients with HE in the prodromal stage may have no evidence of clinical symptoms; therefore, misdiagnosis is possible.

Stage 2: the precoma stage. Patients with HE in this stage usually presents with confusion, sleep and behavioral disorders, and symptoms as described in the prodromal stage further deteriorate. Patients suffer from disorientation and understanding disorders as well as conceptual confusion over time, place, and person. Patients are unable to perform simple intellectual composition (e.g., building blocks, arranging matchstick into pentagon), and have decreased computing capacity (e.g., 100–7 and continuing). Slurred speech, writing disorders, and abnormal behaviors are also common. Sleep perversion and daytime sleep and night awaking may be present. Further, hallucinations, fear, and mania are also observed, and some patients can be misdiagnosed with mental diseases. Patients with liver failure in this stage usually have evident neurological signs such as tendon hyperreflexia, increased muscle tone, ankle clonus, and presence of the Babinski sign. Flapping tremor and an abnormal electroencephalogram can also be observed. Patients may also suffer from uncontrolled muscular activities and ataxia.

Stage 3: the lethargic stage. Patients with HE in the lethargic stage mainly manifest lethargy and insanity, and neurological signs continue and deteriorate. In the majority of time, patients are in a lethargic state, but can be waken up. Patients respond to questioning, but may present confusion and hallucination. Flapping tremor is also present. Muscular tension increases, and there is resistance in the passive limb movements. Pyramidal signs and abnormal waves in EEG can also be noted.

Stage 4: the coma stage. Patients have complete loss of consciousness and are unable to be awakened. In a light coma, patients are responsive to painful stimuli and uncomfortable postures, have tendon hyperreflexia, and increased muscular tension. Patients in this stage are usually unable to co-operate during an examination, and a flapping tremor may not be inducible. In a deep coma, various reflexes disappear; muscular tension reduces; pupils become dilated; and there are paroxysmal convulsions, ankle clonus, hyperventilation, and abnormalities on an electroencephalogram.

Stage of HE is an important indicator of severity of disease. It may reflect not only the severity of brain damage but also the severity of liver disease. It is important to recognize that there is no clear boundary between two neighboring stages and that there might be some overlap between two neighboring stages (therefore missing the middle stage of HE). When the disease condition either deteriorates or improves after therapy, the severity of HE may be reduced by one or two stages.

As mentioned above, the initial symptoms of HE are personality changes. Patients with extrovert personalities (i.e., lively, cheerful) may become depressed, whereas patients with introverted personalities (i.e., withdrawn, reticent) may become euphoric and garrulous. The second most common symptom is a change in behaviors. Initially, patients have sloppy behaviors, such as meaningless behaviors like scattering garbage all over the place and defecating/urinating anywhere, looking at clothes, and touching the bed. Those changes are usually only identified by close observation and careful experience. There are also changes in sleep habits. Patients are often drowsy during the daytime but have difficult sleeping at night or show sleep perversion, which predicts imminent HE. Hepatic foetor is also an important feature of HE. HE patients usually have brain edema and present with nausea, vomiting, dizziness, headache, and either irregular breathing or even apnea. As blood pressure increases there might be a paroxysmal or sustained increase in systolic blood pressure. Bradycardia may be also observed. Muscular tension can increase or the patient can develop a decerebrate posture or even opisthotonus with severe HE. The pupillary light reflex can become blunt/absent, the pupils can become mydriatic, and anisocoria can occur. Achilles and knee tendon hyperreflexia may be observed. It is important to note that some signs might not be obvious in a patient with late-stage HE.

In clinical practice, clinicians may indirectly evaluate the severity of brain edema according to chemosis. Accurate evaluation of brain edema is dependent on the subdural, epidural, or cerebral parenchymal measurement of intracranial pressure. The normal intracranial pressure is <2.7 kPa (20 mmHg), and brain edema is diagnosed once intracranial pressure is >20 mmHg. The most important sign of HE is flapping tremor, which means the presence of HE in stage II. In addition, thinking and intelligence tests (such as number connection test, signature test, mapping test, and computing capability test) are abnormal in HE patients. In some HE patients (especially those with hyperammonemia due to HE), slow waves with high amplitude may be observed on electroencephalogram, and positive-evoked potential is also a characteristic change.

Brain Edema, Cerebral Hernia, and Intracranial Hemorrhage

Brain edema is a complication of ALF. Typical clinical manifestations of brain edema are sustained increase in blood pressure, abnormal pupils, irregular respiration, and papilledema. More than 80% of patients with HE in stage 3 or 4 are likely to develop brain edema, and severe brain edema may result in cerebral hernia. Brain edema has the clinical presentations of increased intracranial pressure and cerebral dysfunction, which can sometimes overlap with the manifestations of HE. It is therefore sometimes difficult to differentiate the two, potentially resulting in misdiagnosis.

HE patients with brain edema may present dysphoria, irascibility, and increased muscular tension, which are more common than in patients with HE without brain edema. If there are concomitant changes in pupils and respiration together with convulsions and/or seizures, cerebral hernia is suspected. In the late stages of liver failure, patients may develop intracranial hemorrhage, causing

respiratory and circulatory arrest and even sudden death. Thus, once cardiopulmonary arrest of unknown cause is present, intracranial hemorrhage should be considered.

Gastrointestinal Bleeding

Concomitant gastrointestinal bleeding in patients with severe hepatitis can be caused by multiple factors, including (1) decreased coagulation factor synthesis by hepatocytes and/or significant inactivation of active coagulation factors in the liver; (2) endotoxemia and disseminated intravascular coagulation consuming a large amount of coagulation factors; (3) hypersplenism causing abnormalities in the quality and quantity of the platelets; (4) portal hypertension causing the rupture of esophageal and gastric varices; and (5) stress response in severe hepatitis leading to diffuse gastric corrosive erosion.

Of the possible complications occurring in liver failure patients, bleeding is the most common and severe. In clinical practice, gastrointestinal bleeding with severe hepatitis seems to make the primary disease worse. It may worsen liver ischemia and hypoxia and aggravate liver dysfunction and ascites. Blood in the gastrointestinal tract can be degraded into ammonia and increase the production of sulfur-like substance, resulting in HE. In addition, bleeding may reduce immune function, which make infections difficult to control. The reduction in effective circulating blood volume may also induce hepatorenal syndrome. Taken together, bleeding may cause multiple organ dysfunction, thereby complicating treatment and reducing the success rate of therapy.

The causes of upper gastrointestinal bleeding are different among patients with different types of liver failure. In ALF and SLF, bleeding is related to reduced synthesis of coagulation-related factors and stress-induced gastric mucosal lesions. In CLF, however, rupture of esophageal and gastric varices and gastric mucosal lesions secondary to portal hypertension are the main causes of gastrointestinal bleeding. In some cases, there is more than one cause of bleeding.

Endotoxemia and Infection

In liver failure, the ability of the monocyte-macrophage system to clear intestine-related endotoxins is reduced significantly, which may lead to intestine-related endotoxemia and deterioration of liver function. This clearly forms a vicious cycle and may cause multiple organ failure if it is severe enough. In addition, patients usually have compromised immune function and are susceptible to infection. Invasive manipulations and use of broad-spectrum antibiotics and immunosuppressants further increase the possibility of secondary infection.

Concomitant infection in liver failure patients has the following characteristics: (1) a high incidence; (2) infection may occur at different sites either simultaneously or sequentially, and abdominal and biliary tract infection is the most common. Once pulmonary infection is present, the disease condition will likely deteriorate, directly causing death; (3) a majority of infections are nosocomial infection, and pathogens are usually resistant to common antibiotics, making therapy challenging; (4) the pathogens causing infection are diverse but mainly Gram-negative bacteria, although

the incidence of Gram-positive and fungal infections is increasing; (5) infection is closely related to the prognosis for liver failure patients. In sum, the more severe the disease, the higher the incidence of infection is and secondary infection may worsen the condition or cause death.

The early diagnosis of secondary infections is based on clinical findings such as signs of infections (i.e., fever, increase in peripheral white blood cells, deterioration of primary disease, specific symptoms of infection of a particular organ). Some patients may not present with an obvious fever and instead only show focal signs of infection. For example, in spontaneous bacterial peritonitis, examination could reveal abdominal tenderness and rebound tenderness and a slight increase in peripheral white blood cells and polymorphonuclear proportion (although they are in normal ranges). In contrast, pulmonary infections can present only with fever while the respiratory symptoms are not obvious/absent and thoracic radiographs fail to show abnormalities in affected patients. In such cases, computed tomography is required to identify the pulmonary lesions. In addition, liver failure patients are vulnerable to fungal infection, especially for those receiving long-term therapy with broad-spectrum antibiotics. Gastrointestinal candidiasis is the most common fungal infection. Oral *Candida albican*s infection is characterized by thickening and a bean residue-like coating on the tongue, gastrointestinal fungal infection is characterized by increased stool frequency and stool with mucus, and pulmonary fungal infection (especially *Aspergillus* infection) is a severe complication of liver failure that can progress rapidly and has a high mortality rate. Once a pulmonary fungal infection is suspected, computed tomography of the thorax should be performed to confirm the diagnosis, and effective antifungal therapy should be initiated as early as possible.

Hepatorenal Syndrome

Hepatorenal syndrome (HRS) refers to progressive functional renal failure in the absence of primary kidney disease in patients with severe liver diseases. HRS is most often diagnosed in the late stages of severe hepatitis and hepatic cirrhosis.

The main clinical manifestations of HRS include:

1. Late stages of liver failure;
2. Renal failure after a reduction in effective circulating blood volume (e.g., water and electrolyte disorder, following paracentesis for ascites, excessive urination due to diuresis, gastrointestinal bleeding, secondary infection, vomiting, and diarrhea). However, HRS may present abruptly with no evident/discoverable causes;
3. HRS is often found in patients with moderate to severe ascites;
4. HRS has no significant relationship with jaundice and HE; and
5. Blood pressure reduces during HRS. Thus, when patients are treated with propranolol for portal hypertension, physicians should pay attention to the baseline blood pressure because reduction in blood pressure after pharmacotherapy may reduce the blood supply to the kidney and decrease glomerular filtration rate, inducing HRS;

6. The abrupt decrease in urine output suggests the presence of HRS. Diuretics usually fail to increase the urine output. Patients often have a reduction in urine sodium and concomitant hyponatremia;
7. Urinalysis shows similarities to prerenal azotemia but displays opposite features to acute tubular necrosis;
8. The symptoms of uremia may overlap with those of liver failure and cause the deterioration of original symptoms. In patients with progressive liver diseases, secondary renal dysfunction is closely related to the deterioration of their general condition, suggesting the aggravation of liver failure. In addition, the presence of uremia may contribute to metabolic complications. Coagulation dysfunction in liver disease patients may be deteriorated due to compromised aggregation of platelets during uremia. Uremia may also aggravate immune dysfunction.

On the basis of clinical characteristics, HRS can be classified into two types. Type I HRS is rare, has an acute onset, and is characterized by progressive renal dysfunction. Serum creatinine may be either 2× that at baseline (i.e., >221 μmol/L or 2.5 mg/dL) within 2 weeks or creatinine clearance decreases by 50% within 24 h (i.e., creatinine clearance of <20 mL/min). Patients with type I HRS have a poor prognosis, with 80% of patients dying within 2 weeks of diagnosis and only 10% of patients can survive for >3 months. The course of disease is short, and symptoms of uremia are not obvious. In type II HRS, which is often found in CLF patients with pre-existing hepatic cirrhosis, has a chronic onset. Ascites patients with type II HRS are usually nonresponsive to diuretics. In type II HRS, renal failure shows a slow progression (lasting for several weeks to months), but the survival rate of patients is lower than that of hepatic cirrhosis patients with ascites. The main clinical consequence is refractory ascites nonresponsive to diuretics in patients with type II HRS. A follow-up study of 234 hepatic cirrhosis patients with ascites showed the accumulative incidence of HRS was 18% within 1 year and 39% within 5 years. A retrospective study showed about 17% of patients with ascites on admission had HRS and HRS patients accounted for 50% of hepatic cirrhosis patients died. However, for hepatic cirrhosis patients, the 2-year and 5-year incidence of HRS is 32% and 41% after development of ascites. A majority of patients (80–95%) die within 3 weeks after development of azotemia.

Hepatopulmonary Syndrome (HPS)

HPS refers to a series of pathophysiological changes and clinical manifestations (including hypoxemia) due to abnormal pulmonary vascular dilation, gas exchange disorder, and abnormal arterial oxygenation. Abnormal arterial oxygenation due to a gas exchange disorder may increase the alveolar-arterial oxygen pressure difference. Hypoxemia is an important pathophysiological basis of HPS, and HPS is a severe pulmonary complication of end-stage liver disease that is clinically characterized by dyspnea and cyanosis.

HPS was first reported by Rydell Hoffbauer in 1956, but it wasn't until 1977 that Kenned and Knudson proposed the full concept of HPS. HPS *per se* refers to pulmonary vascular dilation and the shunting of venous blood with low oxygenation to

arteries in the presence of severe liver disease. HPS is mainly identified in patients with CLF (Child C hepatic cirrhosis). In addition, patients with either acute or chronic liver disease may present with a pulmonary vascular abnormality and arterial hypoxemia. HPS occurs most commonly in patients with hepatic cirrhosis secondary to chronic liver disease, including hepatitis-induced cirrhosis, cryptogenic cirrhosis, alcoholic cirrhosis, and primary biliary cirrhosis, all of which have similar pathophysiological processes as HPS. In HPS, severe ascites, portal hypertension, and arterial hypoxemia ($PaO_2 < 10$ kPa) may be related to the intrapulmonary vascular shunt, excessive production of nitric oxide, lung ventilation-perfusion imbalance, and interstitial fibrosis. The incidence of HPS varies among studies. The incidence of HPS is about 5–29% in chronic liver disease patients but higher in patients with hepatic cirrhosis.

The most common clinical manifestations of HPS are dyspnea, hypoxemia, and cyanosis caused by intrapulmonary vascular dilation and poor arterial oxygenation in the presence of primary liver disease: Patients usually progressively develop respiratory manifestations (e.g., cyanosis, dyspnea, clubbed-fingers/toes, orthostatic hypoxia, supine breathing). Progressive dyspnea is the most common pulmonary symptom of HPS, and cyanosis is a unique and reliable clinical sign. Supine breathing and orthostatic hypoxia are characteristic manifestations of HPS. Pulmonary examination often fails to identify clinically important signs, and HPS is not associated with the either the cause or severity of liver disease. In a fraction of patients with stable liver disease, there is progressive lung dysfunction. Research shows that HPS is associated with esophageal varices and spider angiomas. Intrapulmonary vascular dilation (i.e., pulmonary spider angiomas) is frequently found in liver disease patients with subcutaneous spider angiomas susceptible to hypoxemia. Spider angiomas have been regarded as a marker of extrahepatic involvement.

If patients have no primary heart and lung disease, concomitant lung disease (such as chronic bronchitis, emphysema, pneumonia, and pleural effusion) may coexist with HPS. Affected patients usually have obvious respiratory symptoms; therefore, physicians should differentiate between the conditions. HPS is an independent risk factor for prognosis. Specifically, studies have reported that the median survival time is 10.6 months after the diagnosis of HPS. To date, no effective strategies have been developed for the therapy of HPS. Orthotopic liver transplantation should be performed as early as possible for HPS patients.

Metabolic Disorders

In liver failure, there is massive hepatocyte necrosis that may cause a reduction in glycogenolysis and abnormal gluconeogenesis. Thus, patients are vulnerable to hypoglycemia, shock, coma, and impaired glucose tolerance. The synthetic function of the liver is impaired in such patients, and the serum level of cholesterol and triglycerides decreases. Serum cholesterol has been used as an indicator for the prediction of prognosis of liver failure patients. The frequent use of diuretics can cause water and electrolyte imbalance, of which hypokalemia and hyponatremia are the most common. Such imbalances may also induce HE and brain edema.

Acute Pancreatitis (AP)

AP is a rare, but severe, complication of liver failure. The inciting cause(s) and pathogenesis of AP in patients with viral hepatitis remain unclear but might be associated with viral infection, biliary tract lesions, drugs (steroids and diuretics), and other factors. The reported incidence is 0.2–3%, but one autopsy study shows that the incidence of AP is as high as 33% in patients with severe hepatitis and hepatic cirrhosis. Evidence shows that the incidence of AP is relatively high in patients with advanced liver failure. Further, high serum bilirubin, low albumin, and a significant reduction in prothrombin activity may predict a poor prognosis for AP patients with severe hepatitis and a high mortality.

Two of the following three criteria are required for the diagnosis of AP: (1) patients have abdominal pain characteristic of AP; (2) serum amylase and/or lipase is ≥3× the upper limit of normal; and (3) there are characteristics of AP on medical imaging.

That said, in cases of severe hepatitis with concomitant AP, the symptoms of AP are usually atypical, diverse, and easy to be masked by symptoms of severe hepatitis. Thus, severe hepatitis is often considered the cause of abdominal distension, nausea, and vomiting, even in the presence of AP. In some patients, AP may be misdiagnosed as spontaneous bacterial peritonitis, cholecystitis, or gastritis, which may delay treatment and therefore worsen the patient's condition.

The clinical manifestations of AP are usually atypical in patients with preexisting liver failure, therefore, physicians should highlight the diagnosis of AP in affected patients. When the following findings are observed, AP should be suspected and laboratory and imaging examinations should be performed as soon as possible for the confirmed diagnosis:

1. Patients with severe hepatitis develop abrupt and persistent upper abdominal pain/peritoneal irritation that is nonresponsive to general antispasmodics;
2. Patients present with severe vomiting, severe sialorrhea of unknown cause, and refractory hiccups;
3. Patients manifest repeated and transient episodes of conscious disturbance, which are refractory and not caused by hepatic coma and hypoglycemia-like reaction;
4. Patients have prior chronic cholecystitis or gallstones, receive treatment with diuretics or steroids, and have symptoms and signs described in (1) after exclusion of spontaneous peritonitis.

For patients with severe hepatitis, routine blood testing and urine amylase detection should be performed dynamically. Imaging examinations can be performed simultaneously. Abdominal ultrasonography may be performed within 24–48 h after the onset of abdominal pain, which is helpful for the morphological change in the pancreas and the exclusion of biliary tract disease. However, gas in the gastrointestinal tract during AP may affect the performance of ultrasonography and make accurate diagnosis of AP impossible. Thus, computed tomography is recommended as a standard imaging examination for the diagnosis of AP. Computed tomography

is helpful for the early diagnosis and subsequently timely therapy, which may improve the prognosis.

To facilitate the determination of therapeutic efficacy and the evaluation of prognosis, the Branch of Infectious and Parasitic Diseases and Branch of Hepatology of Chinese Medical Association published the Guideline for the Prevention and Therapy of Viral Hepatitis in 2000 (Xi'an Conference). On the basis of those guidelines, severe hepatitis can be classified as early, intermediate, and advanced severe hepatitis. Specifically, early severe hepatitis meets the diagnostic criteria for severe hepatitis (i.e., severe fatigue, gastrointestinal symptoms, deepening jaundice, serum bilirubin >10 × the upper limit of normal, prothrombin activation of ≤30–40%, or pathological characteristics), but patients have no evidence of HE and no ascites. Intermediate severe hepatitis patients have grade 2 HE or obvious ascites, bleeding tendency (i.e., bleeding point, ecchymosis, and a prothrombin activation of ≤20–30%). Advanced severe hepatitis patients develop refractory complications and HRS, gastrointestinal bleeding, severe bleeding tendency (i.e., ecchymosis at the injection site), severe infection, refractory electrolyte imbalance, HE >grade 2 brain edema, or a prothrombin activation of ≤20%.

1.1.3 Natural History and Characteristics of Different Types of Liver Failure

Currently, some investigators classify the natural history of liver failure into the following: prejaundice stage, bilirubin increase stage, bilirubin plateau stage, and bilirubin reduction stage. Those stages are based on disease progression, serum bilirubin level, and recovery of liver failure patients. In the prejaundice stage, patients have fatigue, anorexia, and an intolerance of oil. They deteriorate gradually, the urine becomes yellow, liver function detection usually shows a significant increase in aspartate aminotransferase and alanine aminotransferase (higher than several thousand), and the prothrombin activity increases. Serum bilirubin increases progressively (i.e., a daily increment of >17.1 μmol/L), and symptoms (fatigue, anorexia) deteriorate after the appearance of jaundice (which is different than manifestations of acute jaundice hepatitis). When the serum bilirubin peaks and remains relatively stable, the disease may be in the bilirubin plateau stage in some patients with no severe complications but present improved mental status and appetite. With the regeneration of hepatocytes, the disease progresses into the bilirubin reduction stage, in which the coagulation, mental status, and appetite improve. When the disease recommences its deterioration, it may progress from the so-called bilirubin increase stage directly to the end stage. In patients with ALF, the bilirubin plateau stage is not obvious, and patients might die shortly after disease onset. If patients survive ALF, the disease may be pathologically classified as a hepatocyte edema type, and liver function will improve in a short period.

Not all types of liver failure (including severe hepatitis B) have clear stages based on their natural history and characteristics, and the respective features are discussed in detail as described in the following sections.

1.1.3.1 Acute Liver Failure (Fulminant Hepatic Failure)

There is still no consensus on the definition of ALF. In 2005, the US Acute Liver Failure Study Group published guidelines for the management of acute liver failure. In those guidelines, they emphasized that liver failure within 26 weeks after onset can be diagnosed with ALF in mother to child transmission of hepatitis B infection (or autoimmune hepatitis), although it has the possibility of progressing into hepatic hepatitis. In addition, some physicians propose that liver failure with an abrupt attack either secondary to chronic hepatitis B or in the presence of other hepatitis virus infection can also be classified as ALF. The pathological basis of ALF may be classified as necrosis- and degeneration-dominant (acute edema) type. In ALF of the necrosis-dominant type, hepatocytes become diffuse and massive necrosis occurs soon after disease onset. In ALF of the degeneration-dominant type, hepatocytes show diffuse and severe swelling.

ALF secondary to acute hepatitis B virus (HBV) infection is rare in clinical practice. Patients with ALF secondary to acute HBV infection usually have no history of HBV infection, are relatively young, and often have predisposing factors (e.g., stress, absence of rest after disease onset, malnutrition, alcoholism, use of liver damaging drugs, pregnancy, concomitant infection). Moreover, it usually progresses rapidly, and patients may develop coagulation dysfunction before the jaundice becomes evident. Such patients present with symptoms of liver failure characterized by HE >grade 2 within 2 weeks, a prothrombin activation ≤40%, an obvious bleeding tendency (i.e., massive petechiae at an injection site), patients have no ascites, disease progresses rapidly and has a poor prognosis, and patients frequently die of complications such as brain edema or cerebral hernia within 3 weeks. Some patients may recover rapidly after appropriate therapy and are usually diagnosed with liver failure of extensive hepatocyte swelling. After recovery, the risk for hepatic cirrhosis is relatively low.

Another situation is the presence of a history of HBV infection in which patients have a good liver condition and no evidence of/mild liver lesions. For HBV patients with ALF, the liver condition is good (as in ALF patients without prior HBV infection) and both ALF patients with and without prior HBV infection share pathological basis, pattern of disease onset, and clinical course.

ALF usually progresses rapidly, and the four stages of ALF (i.e., prejaundice stage, bilirubin increase stage, bilirubin plateau stage, and bilirubin reduction stage) are difficult to identify. ALF may result in high mortality, and a majority of patients directly develop ALF of the bilirubin increase stage or even terminal stage.

1.1.3.2 Subacute Liver Failure (SLF, Subacute Severe Hepatitis)

Pathologically, SLF not only has extensive hepatocyte necrosis but also an obvious inflammatory reaction and formation of regenerative nodules in residual hepatocytes. SLF usually has an origin of ALF. When SLF occurs in patients with or without mild liver lesions, it often shows an abrupt onset. In the early stages, SLF is similar to acute icteric hepatitis and patients progressively deteriorate. Affected individuals may also develop clinical symptoms of liver failure from 15 days to 26 weeks, including severe fatigue, loss of appetite, frequent vomiting, and

deepening jaundice (i.e., a daily increment of >17.1 μmol/L or > 1 mg/dL and an increase in serum bilirubin of >171 μmol/L or 10 mg/dL). Patients usually have hepatic foetor, refractory abdominal distension, ascites (susceptible to concomitant peritonitis), evident bleeding tendencies, and mental and neurological symptoms. In the late stages, hepatorenal syndrome may be present and patients often develop complications (such as gastrointestinal bleeding and hepatic coma) before death. The liver either shrinks or remains normal in size. The course of SLF lasts for several weeks to several months. Patients surviving SLF following therapy usually develop postnecrotic hepatic cirrhosis. Clinically, SLF can be divided into two types. First, the ascites type results in profound jaundice (serum bilirubin of ≥171 μmol/L or > 10 × the upper limit of normal), ascites, and evident bleeding tendencies (i.e., a PTA ≤40%). HE might be absent or present in the late stages. Patients often die of HRS, upper gastrointestinal bleeding, severe secondary infection, and intracranial hemorrhage. SLF of the ascites type accounts for a majority of SLF. Second is the encephalopathy type. Such patients have HE as an initial symptom and present manifestations as in ASH except for course of disease lasting for >14 days. Patients usually die from either brain edema or cerebral hernia. SLF of the encephalopathy type is also not rare.

SLF often has an abrupt onset, and the four stages (i.e., the prejaundice, bilirubin increase, bilirubin plateau, and bilirubin reduction stage) of liver failure are difficult to identify. It is usually associated with a high mortality rate.

1.1.3.3 Acute on Chronic Liver Failure (ACLF, Chronic Severe Hepatitis, CSH)

The pathological basis of ACLF is similar to that of SLF; therefore, they both share clinical characteristics. A majority of patients with ACLF have ascites, spontaneous peritonitis, and biliary tract infection. In the late stages, patients may develop portal hypertension and other complications, repetitive HE and HRS, and most die of gastrointestinal bleeding and HRS.

According to the Guideline for the Prevention and Therapy of Viral Hepatitis (2000), a fraction of patients with CSH meeting the diagnostic criteria can be grouped with ACLF. That is, patients have either chronic hepatitis or compensated hepatitis cirrhosis that remain stable, but some predisposing factors cause the deterioration of liver function, which, thereafter, progresses to liver failure. ACLF refers to acute decompensated liver function in the presence of chronic liver disease. The previously mentioned guidelines emphasize pre-existing chronic liver disease and liver failure due to acute liver dysfunction.

It is important to note that controversy regarding the basis of chronic liver disease persists. In 2002, an English physician proposed that ACLF was diagnosed in chronic liver disease patients with compensated liver function presenting with acute aggravation of liver function within 2–4 weeks due to accidents characterized by jaundice, HE, and/or HRS. German physicians subsequently proposed that the diagnostic criteria for ACLF included (1) the liver has the histological, laboratory, or ultrasound evidence of hepatic cirrhosis; and (2) patients develop jaundice, ascites, coagulation dysfunction, and/or grade 2–4 HE, meeting the definition of

decompensated liver function. The Guideline for the Diagnosis and Therapy of Liver Failure (2006) does not detail chronic liver diseases as a basis of liver failure. However, in general, HBV carrier status may not serve as a baseline liver disease for patients with either chronic hepatitis or hepatic cirrhosis. The term ACLF also highlights that acute or subacute deterioration of liver function occurs, which rapidly progresses to liver failure. Patients often display an abrupt onset and develop severe fatigue and evident gastrointestinal symptoms. In the early stages, there is acute liver damage; therefore, patients usually present with a significant increase in transaminase levels. Thereafter, the disease condition becomes aggravated, and patients may manifest symptoms of liver failure.

ACLF can also be divided into the brain type and ascites type, of which ACLF of the brain type has a higher incidence. Further, the four stages (prejaundice, bilirubin increase, bilirubin plateau, and bilirubin reduction) of liver failure are very clear in patients with ACLF. One goal for physicians and researchers is to determine individualized therapy for ACLF patients according to the specific stage of ACLF.

1.1.3.4 Chronic Liver Failure (CLF, Chronic Severe Hepatitis)

Patients with CLF usually have decompensated hepatic cirrhosis that progressively evolves into chronic liver failure, resulting in clinical manifestations of chronic decompensated liver dysfunction characterized by ascites, portal hypertension, coagulation dysfunction, and HE. The pathological basis of chronic liver failure is hepatic cirrhosis, chronic and progressive aggravation of hepatocyte injury, and reduction in hepatocytes that are unable to maintain normal liver function. Physical examination usually shows signs of chronic liver diseases (such as liver palms and spider angiomas), imaging examination shows characteristics of chronic liver diseases (such as spleen thickening), and laboratory examination also supports the diagnosis of chronic liver diseases (increased gamma-globulin and reduced/inverted albumin/globulin ratio). Of note, a majority of patients have no clear history of liver disease and may initially be misdiagnosed with ALF. Further examinations may provide evidence of hepatic cirrhosis. When patients with hepatic cirrhosis become decompensated, the liver dysfunction usually presents with acute deterioration due to complications or gradually aggravates in a small fraction of patients. On the basis of the above findings, liver failure secondary to decompensated hepatic cirrhosis can be divided into slowly progressive liver failure and acutely deteriorating liver failure. The former shows a chronic status of liver failure and is characterized by repetitive ascites and HE. The latter shows an acute deterioration of liver function in the presence of chronic liver dysfunction, which is similar to ACLF in the disease onset and clinical course. Hepatopulmonary syndrome is often noted in acutely deteriorated liver failure, and patients usually die of heavy gastrointestinal bleeding, HRS, and severe infection.

CLF is generally characterized by slow progression of liver failure, and the course of CLF is relatively long. The four stages (prejaundice, bilirubin increase, bilirubin plateau, and bilirubin reduction) of liver failure are difficult to identify. As such, finding ways to best preserve residual hepatic function reserve is one of the important therapeutic goals in affected individuals.

1.2 Severe Hepatitis/Liver Failure: Diagnosis and Classification

Yu-Ming Wang, Deng-Ming He

Liver failure is a clinical syndrome with high mortality by severe liver damage. It is caused by a variety of causes, results in serious obstacles or decompensation of liver synthesis, detoxication, excretion and biotransformation and appears with coagulation disorders, jaundice, hepatic encephalopathy and ascites as main manifestation. Hepatic failure can be divided into acute liver failure (ALF), subacute liver failure (SALF), acute-on-chronic liver failure (ACLF), and chronic liver failure (CLF). Although the incidence of liver failure is not high in Western countries, the relevant papers, reviews, conferences and other exchanges have increased markedly in recent years. AASLD, EASL and APASL had established a thematic seminar and the definition diagnosis and classification of liver failure has been consistent. At the same time, there are different understandings. Therefore, it is necessary to discuss the main differences of liver failure diagnosis and classification, so as to develop a more rational diagnosis and classification scheme.

1.2.1 Classification of Liver Failure Mechanism

The classification of liver failure involves classification of hepatic injury. A variety of factors (drugs, virus, alcohol, etc.) can cause liver cell damage. Although course and prognosis of liver cell damage are different, the most common mechanism is inflammation. Wieland et al. found that there were two mechanisms of liver cell injury in the immune clearance of HBV; non-soluble cell damage occurring early and soluble cell damage, early mainly non-soluble cell injury, by the study of Gorillas with HBV infected. In 1994, Bonino et al. proposed the theory of non-soluble liver cell damage in the study of Fibrosing Cholestatic Hepatitis (FCH) study. However, this theory was ignored because many scholars believed that it ignored the background of immunosuppression. At that time, FCH was still considered as the injury of endoplasmic reticulum and Golgi apparatus by the excess replication of HBV as well as overexpression of HBV antigen during immune inhibition. However, in 2008, Masayoshi et al. reported that most effective antibodies had been detected in children after living donor liver transplantation who received chickenpox vaccine, attenuated vaccines in children, such as measles, rubella, and mumps. According to this, immune suppression cannot stop antibody production and in FCH, non-cellular immune injury may be present. Recently, we found that there were two types of HBV reactivation in immune-suppressed; high ALT type (>10 × ULN) and low ALT type (<5 × ULN) and both with bad prognosis. We proposed that there may be different injury mechanisms, both immune and non-immune damage. In 2000, Rolando et al. found that 56.8% of acute liver failure patients with systemic inflammatory response syndrome (SIRS), and there was significant

correlation between the progress of hepatic encephalopathy and infection and between the degree of hepatic encephalopathy and the occurred rate of infection.

Infection aggravates degree of illness and fatality rate in liver failure patients. Accordingly, we speculate that serious primary liver injury can cause injury to other organs by cytokines, while injury to other organs can aggravate liver injury. Corresponding with this, we summarized relevant literature and referred that ALF can occur secondary in the development process of multiple organ failure induced by non-primary liver damage. This suggests that this type of liver failure is a special type of liver failure, which is a result of rapid changes in internal environment and inflammatory factors induce liver damage. Therefore, liver failure can be divided into one with primary injury and with secondary injury.

1.2.2 Injury Type and Stage of Liver Failure

Liver failure mainly caused by non-soluble cell injury is extremely rare; as "paralyzed type", "stunned type" or "edematous type", and with the good prognosis. In 2005, small-for-size syndrome after partial liver transplantation was defined by Dahm et al., which is a hepatic failure due to less of liver tissue. Actually, this syndrome can also be seen as a special type of liver failure caused by non-soluble liver cell damage.

Soluble cell injury of liver is relatively common, such as the early hepatic failure in hepatocellular carcinoma after interventional therapy, some drug-induced ALF, etc. Liver failure caused by immune injury is more common in liver failure caused by autoimmune liver disease. Liver failure caused by non-immune injury is more common in liver failure patients with severe cellular immunity damaged (HIV/AIDS patients, chemotherapy patients) or immunity inhibited (in patients after organ transplantation). Primary type is common in fulminant hepatic failure (FHF), the secondary type is seen in severe systemic diseases, such as severe sepsis and acute hemorrhagic.

In fact, clinical liver failure is the result of combination of different proportions of various types of damage factors. Based on the role of various factors in liver failure, it can be categorized. According to acute and sustained, the clinical course and the image changes, liver failure can be divided into different stages with some or certain factors dominated. We have divided it into two categories, necrosis type and the decompensation type. Although this classification is based on practice and clinical management, large-sample analysis is needed in the future.

1.2.3 Classification of Liver Failure

1.2.3.1 Pathophysiology Type

No matter what the cause, liver failure may be divided into two major categories on the pathophysiology. One type is necrosis induced by hepatic inflammation; the other type is decompensation of liver cells. In particular, ALF and SALF are

types of necrosis, ACLF and CLF are decompensated type. Mixed type, both necrosis type and decompensation type, is possible, which treatment should be considered. The relevance of these two type of liver failure mainly reflects in treatment. Necrosis type mainly focuses on treatment of the cause (as antiviral treatment for HBV infection and corticosteroid treatment for autoimmune hepatitis) and symptomatic support treatment (as anti-inflammatory treatment and integrated symptomatic support treatment). Decompensation type mainly focuses on intensive treatment (as control infection for SIRS and control bleeding for gastrointestinal hemorrhage). Some pathophysiological processes such as hepatic encephalopathy (HE) are in both type, but are different in different liver failure type. For example, cerebral edema is more prominent and progressive and less to do with high protein diet in HE patient of necrosis type of liver failure, while is just the opposite in decompensation of hepatic failure.

1.2.3.2 Pathology/Clinical Type

Based on pathologic features and speed of progression, liver failure can be divided into four categories: ALF, SALF, ACLF, and CLF (Tables 1.3 and 1.4). ALF occurs liver failure syndrome characterized with varying degrees of hepatic encephalopathy

Table 1.3 Classification of hepatic failure in Chinese Guidelines

Naming	Definition
ALF	Acute onset, appears hepatic failure characterized by II° or over II° HE in 26 weeks
SALF	More acute onset, clinical manifestations of hepatic failure in 15 days–26 weeks
ACLF	Acute hepatic decompensation based on chronic liver disease
CLF	Chronic hepatic decompensation based on liver cirrhosis

Reproduced with permission from Zhonghua Gan, Zang Bing, Za Zhi, Diagnostic and treatment guidelines for liver failure. Chinese J Hepatol 2006;14(9):643-6 (Article in Chinese) [1]

Table 1.4 Clinical diagnostic criteria for hepatic failure classification ("Diagnostic and treatment Guidelines for liver failure" 2006 version)

Type	Diagnosis	HE
ALF	INR ≥ 1.5 (PTA ≤ 40%) Progressive jaundice in short term, ≤2 weeks	Yes
SALF	TBIL ≥ 171 μmol/L or increased by ≥ 17.1 μmol/L daily PTA ≤ 40%, 15 days–26 weeks	Yes
ACLF	Chronic liver disease TBIL ≥ 171 μmol/L or increased by ≥ 17.1 μmol/L daily PTA ≤40%	Yes or no
CLF	Cirrhosis PTA ≤ 40% TBIL elevation and obvious albumin deterioration Ascites or portal hypertension	Yes or no
CLF	Chronic hepatic decompensation based on liver cirrhosis	

Reproduced with permission from Zhonghua Gan, Zang Bing, Za Zhi, Diagnostic and treatment guidelines for liver failure. Chinese J Hepatol 2006;14(9):643-6 (Article in Chinese) [1]

in 26 weeks except for liver cirrhosis. SALF occurs liver failure syndrome in 15 days to 26 weeks. ACLF is acute hepatic decompensation on the basis of chronic liver disease. CLF refers to chronic hepatic decompensation characterized by ascites, portal hypertension, coagulation disorders or hepatic encephalopathy based on cirrhosis.

Diagnosis and classification of liver failure are the most controversial part, but has tended to unify in recent years. In 2007, Professor Roger William from the University of London proposed the same type criteria as Chinese. The only difference is limited to 8 weeks for ALF and no ASLF. Due to ASLF belonging to ALF, this part is not contradictory for two criteria.

In recent years, a discussion of ACLF proposed many times by Sarin from India made ACLF more valued and accepted. CLF is also getting more recognition. Recently, to clarify meaning and avoid misunderstandings, it was recommended that CLF be converted into end-stage liver failure (ESLF). Although issues related to classification of liver failure have largely agreed, there are some differences in practical application.

Dispute over whether to set up subtypes (namely ACLF/SACLF) of ACLF occurred during drafting the new version of guide. Two suggestions of modification are: (1) With existence of CLD, clinical manifestation of acute (within 2 weeks) and sub-acute (2 ~ 26 weeks) liver function decompensation occur; (2) With existence of CLD, clinical manifestation of acute (within 4 weeks) liver function decompensation occur (this actually is the original version). The reasons are: (1) The classification of 2006 Guide has only been published for 6 years, and it was not easy for it to be widely recognized home and abroad. It requires more time to accumulate experience in this field, so frequent revision are inappropriate; (2) As is generally accepted home and abroad, ACLF mostly occurs within 1 month (4 weeks) after onset, while those as late as 26 weeks after onset are rare (the document of our department indicates the same result); (3) It still requires medical evidence and extensive clinical summary and proof to have the new diagnostic term "SACLF" in English and Chinese established and accepted; (4) Clinical significance and importance of SALF classification are neither prominent nor urgent. After multiple discussions, diagnostic classification in the 2012 Guide applied the latter one (Table 1.5).

Table 1.5 Clinical diagnostic criteria for hepatic failure classification ("Diagnostic and treatment Guidelines for liver failure" 2012 version)

Type	Diagnosis	HE
ALF	PTA ≤ 40% (or INR ≥ 1.5) Progressive jaundice in short term, ≤2 weeks	With
SALF	TBIL≥171 μmol/L or increased by ≥ 17.1 μmol/L daily PTA ≤ 40% (or INR ≥ 1.5), 2 weeks ~ 26 weeks	With/without
ACLF	Chronic liver disease TBIL ≥ 171 μmol/L or increased by ≥17.1 μmol/L daily PTA ≤ 40% (or INR ≥ 1.5)	With/without
CLF	Cirrhosis PTA ≤ 40% (or INR ≥ 1.5) TBIL elevation and obvious albumin deterioration Ascites or other portal hypertension	With

Despite the differences, academia has become unified about the classification of liver failure in the world. The differences towards the convergence of: (1) In terms of naming and classification. Naming has simply become to acute liver failure (including acute and subacute) and CLF (include acute-on-chronic and chronic decompensation), and tend to be more simplified. AASLD guidelines clearly stated that nouns used to differentiate the length of the course (such as hyper acute, acute, and subacute) had claimed not to use. (2) In terms of clinical diagnosis. Because of many cause of liver failure, it is very difficult to achieve unity. The only way is to combine the clinical diagnosis (such as acute hepatitis) and the pathophysiologic diagnosis (such as ALF). (3) If hepatic encephalopathy as a prerequisite for liver failure. Currently, it is a prerequisite for ALF, and not necessary for CLF because hepatic decompensation is the main clinical manifestations.

1.2.4 Stage of Liver Failure

According to the severity of the clinical manifestations, liver failure can be divided into early, middle and late stage.

1.2.4.1 Early Stage

1. Extreme weakness, severe gastrointestinal symptoms such as significant loss of appetite, vomiting and abdominal distension;
2. Progressive jaundice (serum total bilirubin ≥171 μ mol/L or increased by ≥17.1 μ mol/L daily);
3. Bleeding tendency, 30% < prothrombin activity (PTA) ≤40% (or 1.5 < INR ≤ 1.9);
4. No hepatic encephalopathy or other complications.

1.2.4.2 Middle Stage

Based on the early stage of liver failure, further develop to one of the following two:

1. Below grade II hepatic encephalopathy and/or obvious ascites and infection.
2. Obvious bleeding tendency (bleeding point or ecchymoses), and 20% < PTA ≤ 30% (or 1.9 < INR ≤ 2.6).

1.2.4.3 Late Stage

Based on the middle stage of liver failure, further aggravating, severe bleeding tendency (such as ecchymoses on injection site), PTA ≤ 20% (or INR ≥ 2.6), achieve one of the following four: hepatorenal syndrome, upper gastrointestinal bleeding, severe infection and above II° hepatic encephalopathy.

Considering the notorious difficulty to treat hepatic failure and its high mortality rate, special attention has to be paid to and active treatment has to be performed on patients showing the following early-stage clinical features of hepatic failure.

1. Extreme weakness, severe gastrointestinal symptoms such as significant loss of appetite, vomiting and abdominal distension.
2. Progressive jaundice (51 μ mol/L ≤ T.Bil ≤ 171 μ mol/L), and increased by ≥17.1 μ mol/L daily;
3. Bleeding tendency, 40% < prothrombin activity (PTA) ≤50% (or 1.5 < INR ≤ 1.6).

1.2.5 Research Hotspots of Liver Failure Classification

1.2.5.1 The Differences Between ALF and SALF

Clinical practice has shown that, with or without history of chronic liver disease, there are patients with grade II hepatic encephalopathy in a short period, with rapid development, poor prognosis. These patients should be regarded as fulminant type.

Meanwhile, in Asia, including China, there are some patients with severe jaundice, ascites and bleeding as the main presentation, with relatively slow development and very poor prognosis, but without hepatic encephalopathy. These patients should be classified as subacute type.

Fulminant type must have a hepatic encephalopathy. However, it is not necessary for subacute type, which mainly characterized by severe jaundice and ascites. Compared with severe hepatitis in China, fulminant type amounts to acute severe hepatitis and chronic severe hepatitis with acute onset, subacute type amounts to subacute severe hepatitis and chronic severe hepatitis with subacute onset. Currently, a large divergence of these two types is about time, from 10 days to 8 weeks. According to clinical features of ALF, ALF can be further divided into fulminant type and subacute type interval for 4 weeks. However, according to more researches, fulminant hepatitis, characterized by massive necrosis of liver, brain edema, and hepatic encephalopathy, concentrated in 2 weeks, most of them in 10 days or less. Taking into account the subacute type belongs to acute category, subacute are not be established in international classification. ALF are defined as liver failure in 26 weeks.

1.2.5.2 Identification of Acute and Chronic Liver Failure

On the difference between acute and chronic liver failure, most Chinese scholars depend on the past history, which be ignored internationally. The difference lies in this onset. Acute inflammation, necrosis and chronic decompensated were classified as acute and chronic processes, respectively. Most typical example is that patients with acute heavy syndrome of onset in HBV carrier were divided into ALF by the scholars from Hong Kong, Macao and Taiwan. Similarly, Liver failure caused by hepatitis flares in chronic HBV carriers, reactivation of chronic hepatitis B, super infection with HDV and HBeAg seroconversion are included in ALF. It is inconsistent with classification methods in China. The reason lies in greater emphasis on the continuous development processes of hepatitis chronicity and severity in China, and focused on the acute effects this time abroad. Some scholars suggest that considering the significant difference between CLF and the other three types in clinical manifestation, it is worth discussing whether to list CLF as a type of hepatic failure. We believe that significance and importance of CLF classification are: (1) CLF are similar to CRF (uremia) in nephrology and chronic cardiac failure (congestive heart-failure) in cardiology. Although their clinical manifestation differ significantly, the “coexistence of acute and chronic failures” is shared by failures of all those organs; (2) CLF classification has been generally recognized at home and abroad, and the necessity of classification are further proved by the difference between CLF and the other three types; (3) CLF cases are relatively large in proportion (nearly 30%), which is still increasing (since the proportion of ALF/SALF are

lowering); (4) Complications of CLF are common and are found in various forms, with bad prognosis; (5) In CLF patients with correlation to HBV, virus replication are commonly found, which is closely related to decompensation. The efficacy of NUCs are satisfying, which, if taken for a long term, can reverse decompensation, avoiding liver transplantation; it also increases support means in a fast rate, creating more chances for treatment.

1.2.5.3 The Relationship Between the Past History of Liver Disease and Liver Failure

If the strict definition of acute and subacute liver failure as "no past history of liver disease" is executed, how to name the patients who had a history of chronic liver disease (caused by HBV from mother to child transmission in China)? As for ALF and SALF, rigorous definition for the past history of liver disease (including HBV carrying history) is necessary in China, and more interested is in this attack instead of the latent infection in the past, even a dominant attack in Europe and America.

In clinical practice, past history should not be ignored, because patterns of chronic hepatitis B reactivation vary. We summarize them into four types: (1) Burst type: suddenly attack based on immune tolerance state, eventual liver failure; (2) Recurrent type: repeated unequal flares, finally developing into liver failure; (3) Occult type: no obvious attack, presenting with symptoms of decompensation; (4) Document type: Compensated cirrhosis, acute decompensation in certain situations (mainly due to sepsis and other infections). In Burst type absence of history, or only carrier state, the past history can be ignored. In Recurrent type, history of recurrent injury is important, which the significance lies in the extent of the occurrence, duration and consequences. Because these factors determine the basis of injury to the patient's liver, the patients with mild liver disease have mild or even have no hepatic fibrosis and liver cirrhosis, otherwise, the symptoms will be serious and obvious. The former attack often leads to liver necrosis, the latter often lead to decompensation. The difference between occult type and document type is in the speed of decompensation; the former is slow and the latter is fast. In summary, although history has the certain reference value, pathophysiological changes in attack is main of necrosis or decompensation, or a combination of both.

1.2.5.4 The Relationship Between Hepatic Encephalopathy and Liver Failure

Whether hepatic encephalopathy should be considered as a complication of liver failure is controversial, because many scholars have listed it as a prerequisite for liver failure, but in recent years, some patients do not necessarily have encephalopathy. From the complete course and early prevention and treatment of liver failure, it is necessary to incorporate non-encephalopathy type, but the effect and prognosis of the rescue treatment should be divided into the encephalopathy type and non-encephalopathy type, because they are different.

Hepatic encephalopathy is divided into A, B, C type in international guidelines. Type A is acute hepatic encephalopathy (ALFA-HE), which does not include acute hepatic encephalopathy associated with chronic liver disease. Some patients with a

long-term HBV carrier were diagnosed with ACLF or CLF on the first time severe, especially in China. In fact, this type of patients with acute or subacute liver necrosis caused by ALF. The mechanism of hepatic encephalopathy in this type liver failure is different from ALFA-HE, as well as treatment.

1.2.6 Classification and Treatment of Liver Failure

1.2.6.1 Liver Failure Classification and Hepatic Encephalopathy Treatment

Liver failure classification has the greatest impact on treatment of hepatic encephalopathy, based on the following facts: (1) in acute phase of ALF, fasting protein diet on the first day, unnecessary in the short term (4 days); however, chronic hepatic encephalopathy of CLF don't have to fast; (2) the metabolism of branched chain amino acids (BCAA) in ALF was reduced and increased in CLF. This suggests that the former should not be added BCAA, and the latter can supplement the BCAA. (3) high blood ammonia in CLF is more than in ALF. The effect of CLF was better than that of ALF patients, but the effect was not good for deamination drugs. (4) Because cerebral edema in ALF is more than in CLF, it is better to reduce the intracranial pressure in ALF treatment, and in CLF with poor efficacy; (5) as for Type A and C according to the international consensus of hepatic encephalopathy are equivalent to the current ALF and CLF.

1.2.6.2 Classification of Liver Failure and Hypothermia Therapy

Severe cerebral edema has been found in ALF and its mechanism is not clear. Study on cerebral edema treatment have been found that hypothermia therapy can reduce the cerebral blood flow and brain edema.

In 46th EASL, Larsen from Affiliated Hospital of University of Copenhagen in Denmark reported that therapeutic hypothermia did not support application in the treatment of patients with ALF in a prospective, multicenter randomized controlled experimental study (the study was carried out in 2004–2010).

The study results are different from previous studies. We believe that the reason is likely from the bias of group selection. In addition, there is a great difference of the mechanism of hepatic encephalopathy in patients with chronic hepatic failure and ALF, so the response to hypothermia therapy will vary greatly.

Based on the existing research, hypothermia therapy has better effect on hepatic encephalopathy in ALF patients caused by cerebral edema, and has bad effect on hepatic encephalopathy in the chronic decompensated liver failure caused by metabolic abnormalities. As for hepatic encephalopathy in ACLF patients, the effect depends on the roles of cerebral edema in pathogenesis. Therefore, it is recommended that the patients should be carefully screened to obtain a comparable result in the study of the hypothermia therapy in hepatic encephalopathy. We believe that, with the gradual elucidation of the pathogenesis of liver failure, the treatment measures will also be more targeted, the efficiency may be further improved.

1.2.6.3 Classification of Liver Failure and Glucocorticoid Hormones (GCs) Therapy

Glucocorticoids (GCs) therapy in chronic active hepatitis B began in the 1960s to 1970s. However, compared with the control group, GCs did not show better effect. There have been reports that, after stopping the treatment of immunosuppressive therapy, early re-given long-term high-dose GCs can prevent severe hepatitis in HBV reactivation patients. However, this result had not been affirmed in the future clinical practice. Although more application of GCs before 1980s, each effect is different. There is a negative trend in 1990s. We have analyzed, the effect may involve two major factors: one is the choice of indications, dose and duration of treatment; another is the prevention of adverse reactions and complications of GCs.

According to the guidelines for diagnosis and treatment of liver failure (2006) in China, liver failure without viral infections, such as autoimmune hepatitis and acute alcoholism (severe alcoholic liver disease), etc. are GCs indications. At the early stage of liver failure caused by other reasons, in the patients with developed rapidly and no serious infection, bleeding and other complications, GCs may be appropriate to use. GCs can improve the survival rate of patients with autoimmune hepatitis and severe alcoholic hepatitis, and has been recognized by most scholars. For hepatitis flares in CHB patients, if on the basis of combined application of NUCs, GCs will inhibit excessive hyperactivity of host cellular immunity and excessive release of cytokines, and help preventing liver cell death. At the same time, the role of GCs in the treatment of CHB and the specific usage, as well as the effect of combined treatment is still controversial. The reason is that the advantages and disadvantages of GCs are very prominent. The key of success or failure lies in the clinical skills.

In recent years, the reports of GCs for the treatment of HBV related liver failure increased. There are three main reasons: (1) NUCs can effectively resist HBV replication due to GCs; (2) application of proton pump inhibitors can effectively prevent gastrointestinal bleeding due to GCs; (3) increasing of infection prevention and treatment can effectively fight infection due to GCs.

Even so, the above three aspects of the problem have not been satisfactorily resolved. Therefore, we put forward several viewpoints on the current application of GCs: first, to fully analyze the advantages and disadvantages, to consider the main function and purpose after the GCs application and the risk of adverse events before expanding the indications of GCs. Secondly, both short course treatment (3–5 days) and long course treatment has drawbacks. The former may not be sufficient to adequately inhibit a strong immune response, and induce a stronger immune response after a sudden stop; the latter can induce bleeding, infection or viral resistance. Finally, in a common clinical liver failure induced by HBV reactivation under immunosuppression, the mechanism is often unrelated with immune activation. The typical representative is FCH, and the prognosis is extremely bad. It should be vigilant, focus on prevention. Our department has treated a chronic hepatitis C (CHC) patients after kidney transplant and taking large doses of GCs. Severe FCH was induced, resulting in liver failure and eventually died.

1.2.6.4 Classification of Liver Failure and Hepatic Stem Cell Therapy

In recent years, the hepatic stem cell therapy of liver failure was concerned, human stem cell transplantation in the treatment of clinical study on severe hepatitis/liver failure were carried out and the results were satisfactory. However, due to the difference of the patient's condition or stage, and most of which are case report, it is difficult to draw an objective conclusion.

We have repeatedly reported that the human umbilical cord blood stem cells and bone marrow stem cells in vitro and in vivo can be successfully transformed into liver cells, but the biggest obstacle to its application to clinical practice is the limited number. It is difficult to repopulate the whole liver or at least part of liver compensatory function.

In addition, considering the part liver failure patients with cirrhosis background, which clinical manifestations as ACLF and CLF in the practical application, there are the following issues: (1) It is difficult to provide effective growth and functional support for implanted cells in a diseased liver; (2) the original portal hypertension can be aggravated by portal vein implanted cells; (3) input cells by vein can obstruct the pulmonary vein, thus affect the pulmonary circulation; (4) if the implanted cells for allogeneic or xenogeneic tissue, may have compatibility problems; (5) when using GCs to prevent rejection, severe hepatitis patients are easy to infection and other related adverse reactions.

1.2.7 Problems and Prospects

Finally, it is pointed out that there is a tendency of the academic circles to the understanding of liver failure for decades, that is, the existence of the chronic process is often neglected in the process of analysis, and vice versa. A typical example, until nearly a few years ago, many scholars in the world still do not recognize the existence of ACLF and CLF. Chinese research papers on liver failure published very little in the international, the main reason is that the ACLF and CLF is not clearly defined. The papers are often rejected because the diagnosis of type does not conform to the international. In contrast, as previously mentioned in the definition of AKI and HRS, the existence of ALF (including SALF) is ignored. Another example: in the report of antiviral treatment of HBV related liver failure, some foreign scholars put forward that the liver failure should be changed to the decompensated liver cirrhosis, the reason is that acute HBV infection is self-limited.

However, at present, there has been a clinical recognition that the HBV related ALF is actually an acute episode of chronic carrying process and should be referred to as ACLF. In Chinese guidelines for the diagnosis and treatment of liver failure (2006), liver failure was divided into four types, mainly because of their different mechanisms and treatment (sometimes even the opposite). Due to limitation of the space and data, we have not discussed infection, bleeding and other complications in relation to liver failure. These pathological processes are closely related to the classification, and should be studied further in relation to liver failure. In future, we

should develop classification of liver failure according to the mechanism of liver injury with different causes, and provide the basis for clinical types of hepatic failure. In order to improve the level of diagnosis and treatment of liver failure, the mechanism based classification should be carefully assessed and evaluated.

1.3 Pathological Features of Acute Exacerbation of Chronic Hepatitis B and Severe Hepatitis B (Liver Failure)

Jing-Ming Zhao

Acute Exacerbation of Chronic Hepatitis B (AECHB) is the clinicopathological manifestation of aggravation of liver necroinflammation and disease deterioration in HBV-infected patients. When serum ALT levels increase by 5 times above upper limit of normal (ULN), it is a severe incident of poor prognosis of hepatitis B. Severe hepatitis B (liver failure) is the extreme clinicopathological manifestation of exacerbation of HBV infection, and it has a high clinical mortality. AECHB can be caused by excessive immune response to HBV infection, induced by factors such as virus replication, anti-viral resistance and non-standard anti-viral therapy or resulted from Overlapping factors including human immunodeficiency virus (HIV) infection, hepatitis C virus (HCV) infection, hepatitis E virus (HEV) infection and drugs, toxins and alcohol-induced liver injury. Liver biopsy is considered to be a 'Golden Standard' of definite diagnosis, severity evaluation and treatment effect assessment, and it is irreplaceable compared with other examinations. Histopathological evaluation of AECHB and liver failure not only contribute to the definite diagnosis of severity of AECHB and provide pathological evidence for effective clinical treatment of hepatitis B, but also is helpful to the early detection of histopathological proof of AECHB by pathological examinations and of pre-warning function for clinical treatment of AECHB. This section mainly focuses on pathological features of AECHB and other types of liver failure.

1.3.1 Pathological Features of AECHB

AECHB, clinicopathological manifestation of aggravation of liver necroinflammation and disease deterioration of chronic hepatitis B, tends to be of poor prognosis without positive and effective intervention. Its pathological characteristics mainly include: ballooning degeneration of diffuse hepatocytes, significantly increased focal necrosis, confluent necrosis, bridging necrosis, extensive and intensive interface hepatitis, massive or submassive necrosis, many neutrophil infiltrations in hepatic lobules and portal areas and moderate intrahepatic cholestasis. Prominent hepatocyte necrosis is the pathological foundation of AECHB and manifests extensive multifocal necrosis, confluent necrosis, bridging necrosis and other forms of necrocytosis. Severe ones can even occur submassive and massive necrosis leading to the extreme form of AECHB, severe hepatitis (liver failure).

1.3.1.1 Ballooning Degeneration of Diffuse Hepatocyte

Ballooning degeneration of diffuse hepatocytes is one of pathological characteristics of AECHB (Fig. 1.1). The hepatocytes manifest sparse and granular cytoplasm, sometimes can be micro-bubble like. The degenerated hepatocyte is 2–4 times bigger than the normal hepatocyte. Sometimes the ballooning degenerated hepatocytes can fuse and transform into multinucleated cells, and this lesion is similar to that of neonatal giant cell hepatitis when it is relatively extensive. The general performance of the liver is increased volume, tense capsular and cutting edge eversion due to tension. Ballooning degeneration of hepatocyte is not specific histological manifestation of hepatitis Band also can occur in liver tissues of hepatitis caused by factors such as alcohol or drugs. Extensive and diffuse ballooning degeneration of hepatocyte can make the hepatocytic plate wider, and hepatic sinusoid is pressed to be narrower, causing microcirculation disorder of liver tissue and exacerbation of disease.

1.3.1.2 Significant Increase in Multifocal Necrosis

Liver lobular inflammation activity is enhanced, and apoptotic bodies and focal necrosis increased significantly when AECHB occurs. Hepatocyte apoptosis is the programmed necrosis and one of the major forms of hepatocyte death in HBV infection. Histopathology manifests cell membrane shrinkage, deepened cytoplasmic staining, eosinophilic degeneration. Free apoptotic bodies in liver sinus are large or the apoptotic cell fragments, sometimes containing nuclear fragments. Focal necrosis is another form of liver cell necrosis and manifests as an interruption of the hepatocytic cords or replacement by focal lymphocytes and macrophages, with hepatic regeneration that often causes irregular arrangement of hepatocytic cords. This necrosis often inferred from the disappearance of the hepatocytes and the infiltration of inflammatory cells rather than what is actually seen under a microscope.

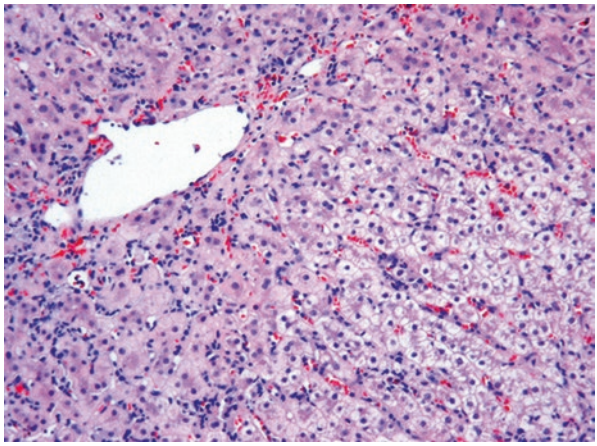

Fig. 1.1 Ballooning degeneration of liver cells (HE staining, 200 times). Swelling hepatocytes presented increased size and loosing cytoplasm, further develop to ballooning degeneration showed almost spherical in size and transparent cytoplasm, predominantly in the lower right

1.3.1.3 Confluent Necrosis and Bridging Necrosis

Confluent necrosis and bridging necrosis are the common histopathological changes, play important roles in the progression of AECHB and are closely related to the adverse prognosis of hepatitis B. Due to the larger necrosis range of confluent necrosis and bridging necrosis, even during repair stage after going through the active stage, the liver tissue often undergoes fibrous repair, resulting in liver fibrosis and hepatic lobule reconstruction, thereby causing liver cirrhosis. Statistics show that about 18% of the patients with viral hepatitis who had bridging necrosis progress to cirrhosis.

Confluent necrosis is regional lytic necrosis of hepatocytes on a larger scale and is common in active stage or aggravation of viral hepatitis, or drug-induced liver injury, which often occurs around the central veins and inflammatory cell infiltration is not obvious. Specific confluent necrosis can also be seen in other parts. Take ferrous sulfate poisoning for example, confluent necrosis is more common in zone 1 of liver acinus, and when confluent necrosis expands to connecting vascular or portal area, bridging necrosis occurs (Fig. 1.2). Bridging necrosis is large area hepatic lytic necrosis that connect the portal area to central area (P-C), portal area to portal area (P-P), and central area to central area (C-C). It can be caused by the expansion and confluence of interface hepatitis, or a one-time large-scale translocular necrosis. P-C necrosis: it starts on the periphery of the lobules, affects the central hepatic lobules when it expands, and forms bridging necrosis phenomenon. The currently acknowledged mechanism is as follows: the initial pathological change is serious periphery necrosis of hepatic lobules. With the aggravation of the disease, microcirculatory disturbance occurs in the lobules and causes the hypoxia, degeneration and necrosis of central area liver cells. P-P bridging necrosis: most scholars think it is caused by the expansion of interface inflammation, especially based on the fibrous septum, HBV load increasing significantly, immune response enhancing, activating the signal pathway of liver cell death. With the enhancement of the lesion activity, fresh and severe necrosis occurs. C-C bridging necrosis: it is usually seen in serious disease with bridging necrosis of hepatocytes, but mononuclear cells

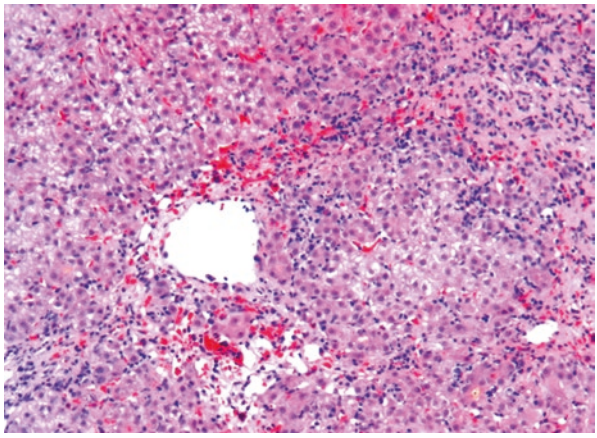

Fig. 1.2 Confluent necrosis and bridging necrosis (HE staining, 200 times). Zonal dissolved necrosis of liver cells around the central vein, known as confluent necrosis (Black arrow), when expanded to connect central vein to portal tract, bridging necrosis occurred (Green arrow)

infiltration is rarely seen in the necrosis area and serum transaminase increases significantly (up to 1000 IU/L above).

Histological manifestations of bridging necrosis can vary due to the different stages of the disease. In the early stage, the liver parenchymal cells necrotize and then disappears, reticular framework residue accompanied by the infiltration of lymphocytes and macrophages. With the time extending, reticular framework collapses and forms the sparse interval crossing the liver tissues. When bridging necrosis is accompanied with reticular framework collapse, with hepatic necrosis and regeneration, disorder of hepatic lobules occurs. At this time, it is difficult to distinguish the fibrous septa between bridging necrosis and chronic hepatitis, and elastic fiber staining can help to solve this problem. The elastic fiber staining of bridging necrosis is negative, because elastic fiber formation often takes several months.

1.3.1.4 Extensive and Intensive Interface Hepatitis

Interface hepatitis, formerly known as piecemeal necrosis, is one of the symbolic histological manifestations in the chronic activity of chronic hepatitis B. It mainly refers to single or small clusters of liver cells around the portal areas necrose and shedding, leading to worm-eaten defect of limiting plates. Significant lymphocytes infiltration is commonly seen in and around the portal area. Mononuclear cells extend to the hepatic lobules along the destructive limiting plates and encase the necrotic liver cells, resulting in the enlargement of portal areas (Fig. 1.3). Interface hepatitis increases significantly and extensive interface hepatitis occurs in AECHB. The interface hepatitis area can exceed 50% of portal areas periphery and be more than a third of the depth of hepatic lobules, even causing bridging necrosis (P-P and P-C). Because the limiting plate of the lobules is an important structure to maintain a whole hepatic lobules, interface hepatitis destroys the integrity of hepatic lobule structure. Extensive and intensive interface hepatitis can often cause bridging necrosis and bridging fibrosis, and is an essential part of poor prognosis of AECHB.

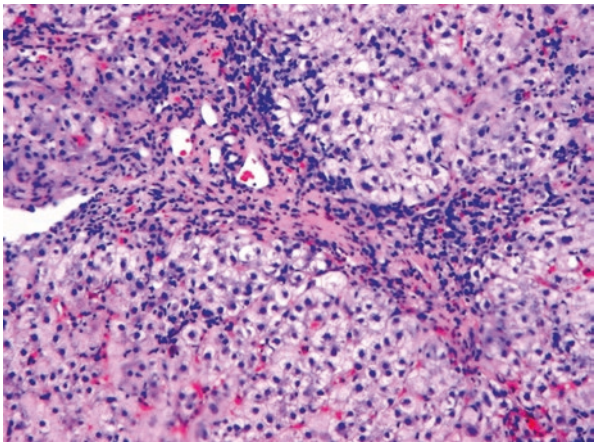

Fig. 1.3 Interface hepatitis (HE staining, 200 times). Severe interface hepatitis showed marked lymphocytic infiltration along the interface in ‘insect-bite’ shape

1.3.1.5 Massive Necrosis and Sub-Massive Necrosis

Massive necrosis and sub-massive necrosis are considered to be the basic pathological changes of severe hepatitis (liver failure) and major substratum for histologic diagnosis of liver failure. Massive necrosis is the diffuse lytic necrosis of the liver parenchyma involving more than 2/3 of hepatic lobules. Thorough and rapid liver tissue necrosis is shown with invisible necrosis process, and only reticular framework remained and is filled with red blood cells (Fig. 1.4). Sub-massive necrosis is diffuse liquefaction necrosis of the liver parenchyma involving 1/2–2/3 of hepatic lobules. Reticular framework collapses and forms reticular fiber bundles, residual liver cells and bile ducts proliferate. Massive necrosis and sub-massive necrosis will seriously affect the prognosis of patients with high fatality rate once they occur. The cause of massive necrosis and sub-massive necrosis remains unclear, and the possible causes include excessive virus replication, virus mutation, overlapping with other virus infection and microcirculation, etc. When extensive confluent necrosis, massive necrosis and sub-massive necrosis involve the entire hepatic lobule and even several adjacent hepatic lobule, causing a lobular or adjacent several lobular hepatocytes lytic necrosis, then the panacinar or multiacinar necrosis occur, which is the most severe form of necrosis of AECHB. In panacinar and multi-acinar necrosis, with large range of liver cell necrosis, only a small amount of liver cells remain. Residues of clump, rosetting, island or glands-like arrayed liver cells are commonly seen around the collapsed reticular framework after necrosis or loose fibrous connective tissue. Due to distortion, normal structure cannot be recognized and sometimes can only be identified by portal area range around the necrotic area. Cells proliferation can be observed in periportal area, arraying like bile duct structure and these cells can express hallmarks of hepatocyte and bile duct epithelial cells at the same time, which is considered to be the histological manifestation of liver stem cells (hepatic stem cell) activation and proliferation. Infiltrating inflammatory cell types tend to be multiple, and the quantity varies. When there is less infiltrating inflammatory cells, the main cell type is macrophage, often containing brown pigment particles. Notably, the liver biopsy might be error in diagnosing the necrosis of

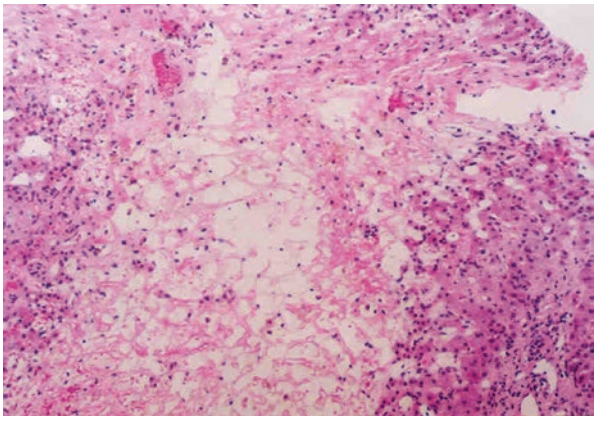

Fig. 1.4 Massive necrosis (HE staining, 200 times). Wide range of diffuse dissolved necrosis of liver parenchyma (above 2/3 lobule), with only the mesh stents remained

the whole lobules and multi-lobules, and that is due to limited amount of liver biopsy specimens. For example, multi-acini necrosis occurs only in the area under the liver capsule, and liver biopsy pathological examination may overestimate the severity of illness.

1.3.1.6 Neutrophils Infiltration in Hepatic Lobules and Portal Areas

In AECHB, types of infiltration inflammatory cells in the liver tissue are various, and most of them contain many neutrophils. It is different from obvious lymphocyte accumulation within liver parenchyma and portal area common seen in hepatitis B. Usually, CD8 + T cells/Cytotoxic T lymphocytes (CTLs) are the major effector cells of the inflammatory response in hepatitis B. But when exacerbation occurs, the neutrophils in inflammatory cells infiltrating liver acinus and portal area increase significantly. Neutrophils play an important role in innate immunity, and are the first inflammatory cells migrating to the lesion during inflammatory response. These neutrophils kill the invading pathogenic microorganisms through releasing protease and anti-microbial proteins, and producing reactive oxygen. Meanwhile, neutrophils have important functions in activation and regulation of innate immunity reaction and adaptive immunity reaction and could release cytokines such as IL-8 to participate in regulating adaptive immunity reaction. Although cytokines produced by neutrophils are less than mononuclear macrophages, since neutrophils are the first inflammatory cell moving to inflammatory lesion, the immune regulator function of neutrophils is more important during the early or acute stage of immunity reaction. Evidently increased neutrophils in liver tissues of AECHB might be beneficial for eliminating infected cells, but it is also a "double-edged sword". Accumulation of neutrophils may cause extreme immune response and excessive inflammatory reaction might lead to deterioration. Therefore, when increasing neutrophils appear in liver tissues of hepatitis B patients, more attention should be paid to identify whether virus mutation, overlap infection, drug-induced liver injury occur and cause AECHB.

1.3.1.7 Moderate or Severe Intrahepatic Cholestasis

Intrahepatic cholestasis (cholestasis), especially moderate or severe cholestasis, is one of the common histologic manifestations of AECHB. Intrahepatic cholestasis takes shape from the bile thrombus within the cholangioli around the central vein which is hard to be identified, forming cholestasis in expansive interlobular bile duct and large "bile lake" in hepatic tissue (Fig. 1.5). Microscopically, bile can be characterized by dark brown, green or yellow color, occasionally also can present the gray which is difficult to recognize. Bilirubin is revealed as green in Van Gieson staining, which is helpful to pathological diagnosis of intrahepatic cholestasis. Moderate and severe intrahepatic cholestasis often cause feather-like degeneration of hepatocytes, even intrahepatic cholestasis infarction. With the extension of duration of intrahepatic cholestasis, the inter-hepatocyte structure relation of 2–3 normal liver cells surrounding the capillary bile duct also changes. The number of liver cells around the bile duct increases, and the bile canaliculi expands, causing the cholestasis related rosette structure forms and the fusing multi-nucleus giant hepatocyte

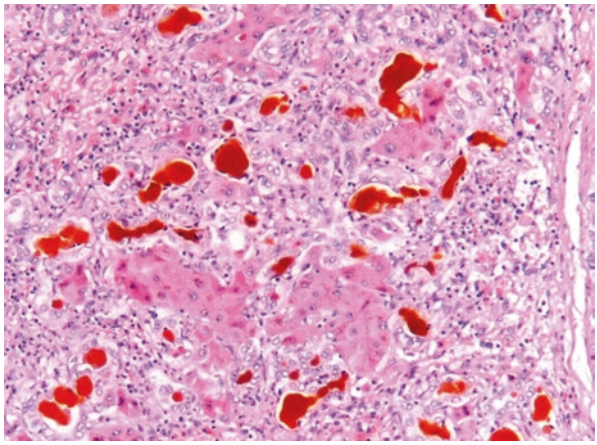

Fig. 1.5 Intrahepatic cholestasis (HE staining, 200 times). Severe intrahepatic cholestasis formed 'bile lakes'

appears. Kupffer cells with brown bile pigment can be seen in the hepatic sinusoid. Remarkably, although intrahepatic cholestasis is often associated with clinical symptoms such as jaundice and risen serum bilirubin, the severity of intrahepatic cholestasis is not consistent with clinical symptoms and serum bilirubin level.

1.3.2 Pathological Features of Severe Hepatitis B (Liver Failure)

Severe hepatitis B (liver failure) is the most severe liver syndrome complex, which is characterized by poor clinical course and high mortality rate. Over the years, scholars have continued to explore the definition, classification, diagnosis and treatment of liver failure, but so far no consensus has been reached. According to histopathological features and progression of the disease, China released *Guidelines on Diagnosis and Treatment of Liver Failure* in 2006, which divided liver failure into four categories: acute liver failure (ALF), sub-acute liver failure (SALF), acute-on-chronic liver failure (ACLF) and chronic liver failure (CLF). HBV infection is the most common cause of liver failure in China.

1.3.2.1 Acute Liver Failure

Acute liver failure is characterized by acute onset, and hepatic encephalopathy (above stage II) often develops within 2 weeks of the onset, which results in high mortality rate. Former knowledge about acute severe hepatitis mostly comes from the autopsy. Nowadays, with the development and universal application of biopsy technique, and the further study on acute liver failure, we have better understanding of the development and process of necrotic lesions, and can predict prognosis and outcome according to the necrotic area and type.

In ALF, liver atrophy is significantly present in gross pathology inspection, especially for the left lobe. Coverings shrinkage, thin edge, soft liver texture, section may be yellow or reddish-brown, and some area is red alternating with yellow, and the weight of the liver drops sharply to 600–700 g. Histopathology emphasizes

extensive and consistent liver cell necrosis caused by one-time strike, and most patients die in the short term. The morphology of liver tissues is relatively simple, manifested as massive or sub-massive necrosis of liver cells, dissociation of liver cords and hepatolysis. The regeneration of liver cells is not obvious, and surviving liver cells show clear ballooning degeneration. Hepatic sinus expand and, congest with blood and occur hemorrhage. Kupffer cell proliferates and sinusoidal mesh stent does not collapse or completely collapse. Quantity of liver cell necrosis is closely correlated with prognosis. If the amount of necrosis is over 70%, mostly the patient will not survive. If the amount is less than 50%, the patient is expected to resume with rapid regeneration of hepatocytes. If there is diffuse small steatosis, the prognosis is often poor.

Concerning the hepatic regeneration of acute liver failure, former pathology emphasizes on liver cell and bile duct cell proliferation of sub-acute liver failure, and relatively neglects cell regeneration of acute liver failure. Based on the authors' knowledge, in some acute liver failure cases, liver tissue demonstrates obvious bile duct-like or acinar-like regeneration within 4 days after onset. The regenerated liver cells were co-expressing albumin and CK18, CK19, indicating these cells have double markers of hepatocytes and biliary epithelial cells, which presumably come from liver pluripotent stem cells. The regenerated liver cells in acute liver failure have their unique characteristics that degenerated and regenerated cells co-exist in the liver. As the time of liver biopsy is different, the morphological change is also varied because of the rapid restoration. The usual dual-core, large nuclear or nuclear fission are rarely seen in regenerated liver cells; liver cell body swelling, transparency of cytoplasmic periphery and slightly basophilic center are commonly seen in regenerated liver cells.

Because of cell enlargement and transparent cytoplasm, it is often difficult to distinguish from serious ballooning degeneration; in some cases, it shows bile granules within the cytoplasm, bile thrombus within bile capillary with cell swelling and transparency symptoms, which is also similar to the feather-like degeneration caused by bile salt siltation. However, unlike feather-like degeneration which was scattered by small groups or disorderly arranged severe ballooning degeneration, cell enlargement often shows the pole adenoid arrangement, which is known as a sign of liver cell proliferation. The outcomes of continuous liver biopsy also prove its rapid regeneration. Nayak et al. also proposed that hepatocyte swelling during the acute liver failure recovery stage indicates good prognosis. The continuous liver biopsy of 12 liver transplant centers in Europe also confirmed that the appearance of liver cells enlargement after 12 days of partial liver transplant of acute liver failure with massive necrosis, cells linked to sheets, and lobular structure are basically recovered in 2 to 3 months. The vacuolation, cholestasis and duct-like structure presented by these regenerated enlarged liver cells will be gradually disappeared.

1.3.2.2 Sub-Acute Liver Failure

The onset of SALF is acute, and liver failure syndromes appear within 15 days to 26 weeks, mostly caused by delay of acute liver failure. In SALF, liver atrophy is mainly presented as in gross inspection, and variable sizes of regenerative nodules,

the yellow-green cutting surface due to cholestasis. Histopathology manifests the new and old sub-massive necrosis of liver tissues, or bridging necrosis. In older necrosis area, reticular fibers collapse, or collagen deposit. Survived liver cells may have varied degrees of regeneration, and are arranged in nodular. Fine, small bile duct proliferation and cholestasis are commonly seen in the periphery lobe. The sinusoids congest in the early stage, collapse in mid-stage, and occlude in late stage.

The histopathological distinction of SALF and ALF is based on the consistency of necrotic lesions. ALF emphasizes consistency of necrotic lesions, that is, 'one-time strike', while the necrotic lesions of SALF are mixed, caused by 'multiple attack'. In addition, differences also exist in aspects such as cell regeneration and extracellular matrix (ECM) expression between ALF and SALF. Liver stem cells play an important role in the liver cell regeneration process of ALF. The regenerated liver cells express dual markers of liver cell and bile duct cell, and are often orderly proliferated along mesh stents. Whereas SALF presents unipolar regeneration of liver cell and bile duct cell, and the regenerated liver cells is disorderly arranged. Due to the different length of disease course, there is no obvious ECM deposit in ALF, while SALF presents in III collagen-based ECM deposit.

This type of liver failure often develops into post-necrotic cirrhosis.

1.3.2.3 Acute-On-Chronic Liver Failure (ACLF)

Acute-on-chronic liver failure (ACLF) refers to acute liver function decompensation occurring on the basis of chronic liver disease.

Liver gross manifestation of acute-on-chronic liver failure differs with the different stages of chronic liver disease. For instance, ACLF occurring in the stage of cirrhosis is accompanied by hepatic cirrhosis nodules besides liver atrophy. The main histology of ACLF is new and varied degrees of liver cells necrosis, hepatocyte focal and spotty necrosis, bridging necrosis, confluent necrosis, massive necrosis and sub-massive necrosis on the basis of chronic liver injury. The common chronic changes are as follows: fibrosis in collapsed reticular framework or periportal area with obvious extracellular matrix deposit, forming of large number of fibrous septa, sparse scars, or bridging fibrotic septa when distortion of lobule structures associated with disproportionate numbers of central veins and portal area, pseudolobule formed; twin-cell or multiple-cell of liver plate is commonly seen and the liver plate lose the radiated array, activated regeneration of liver cell caused the occurrence of tumor-like cell.

1.3.2.4 Chronic Liver Failure (CLF)

Chronic liver failure is chronic liver function decompensation caused by progressive deterioration of liver function on the basis of liver cirrhosis, with ascites, portal hypertension, blood coagulation dysfunction and hepatic encephalopathy as main symptoms.

Liver gross appearance of CLF is significant liver atrophy and nodular liver cirrhosis. Histopathological changes are mainly those of liver cirrhosis, including diffuse liver fibrosis, nodular liver cirrhosis with unevenly distributed liver cells necrosis.

1.3.3 Pathology Features of Exacerbation of Special Hepatitis B

Progression of hepatitis B is an interaction between HBV infection and body response. Development of AECHB is mainly caused by obvious increased viral load and/or decreased immune clearance. Large number of HBV replication can activate hepatocyte death pathways, leading to serious liver inflammation, necrosis and aggregation of disease. Additionally, HBV infection overlapping with HCV/HIV, or with etiological factors like drugs and ethanol, could also affect disease progression.

1.3.3.1 Fibrosing Cholestatic Hepatitis

Fibrosing cholestatic hepatitis (FCH), a new clinicopathological type, develops in stages of severe immunosuppression caused by various reasons, especially in hepatitis virus-infected patients lots of immunosuppressant after organ transplant. Due to immunosuppressor, HBV replicates rapidly in the patients, leading to quick progression of hepatitis and progressive failure of liver function.

The histopathological features of FCH are as follows: fibrosis straps starching from the portal area to hepatic sinusoid and circumvoluting basal plates of biliary epithelial; obvious intrahepatic and hepatocytes cholestasis, bile embolism forms in small bile duct; hepatocytes ballooning degeneration with disappearance of cells; mass ground-glass hepatocyte; mild to moderate mixed inflammatory reaction (Fig. 1.6).

FCH could quickly proceed to liver failure with blood coagulation dysfunction and hepatic encephalopathy, and mostly die in several weeks to months.

1.3.3.2 Coinfection of Hepatitis B and Other Viruses

Due to common transmission, coinfection with HBV and HCV or HIV is not rare clinically.

5–20% of the chronic HBV infected patients carry HCV antibody and there are 7–20 million coinfectious patients all over the world. Studies showed that HBV and

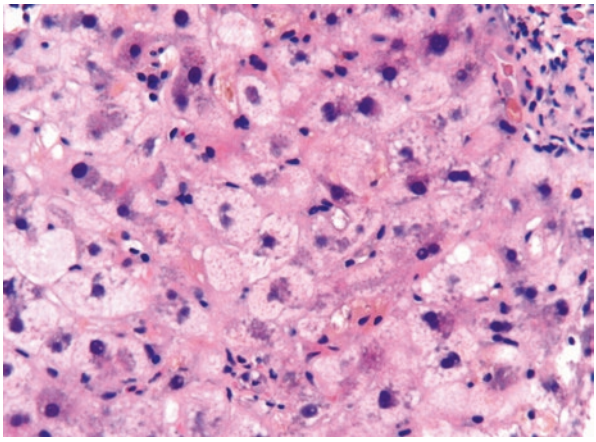

Fig. 1.6 Fibrosing cholestatic hepatitis (HE staining, 400 times). Notable intrahepatic cholestasis (black arrow) and ballooning degeneration and/or feathery degeneration (red arrow), a large number of ground glass hepatocytes (blue arrow), associated with inflammatory response (green arrow)

HCV coinfection could promote the synthesis of collagen and promote disease progression to liver fibrosis. Compared to the single HBV infection, HBV and HCV coinfection presents more severe liver fibrosis and inflammatory necrosis. Studies demonstrated that HBV and HCV coinfection could promote CHB progression, cause severe damage of the liver function and then exacerbation, increasing the probability of liver fibrosis, liver cancer and liver failure in CHB patients.

In HBV and HIV coinfection, HIV infection can affect the natural history of HBV and accelerate the development to end-stage liver disease and liver cirrhosis. Immune deficiency induced by HIV infection fosters HBV replication, and even fibrotic cholestasis hepatitis in severe cases. Histologically, CHB caused by HBV and HIV coinfection had a severer fibrosis than that by simple HBV infection.

1.3.3.3 Hepatitis B Overlap Drug or Alcohol Induced Liver Injury

Cases of hepatitis B overlapping drug or alcohol induced liver damage are not rare. Even antiviral drugs can cause AECHB, and there is previous case report on hepatitis B patient died of acute liver failure induced by anti-hepatitis B virus medication lamivudine. Abuse or nonstandard drug use and alcoholism have become the common causes of AECHB. Pathology manifests features of overlapping drug or alcohol induced liver injury on the basis of hepatitis B changes. For instance, in AECHB caused by overlapping drug-induced liver injury, liver tissues present histological characteristics of hepatitis B accompanied with drug-induced liver injury, such as evident increased percentages of infiltrated eosinophils and neutrophils in liver tissues, confluent necrosis with less inflammatory cell infiltration in acinus three area, cholestasis of bile canaliculi and so on.

In summary, AECHB has its relative histopathological features. Understanding of these pathological characteristics can not only help with clinical diagnosis and effective treatment, but also aid to prevent AECHB. It is important to note that despite the value of histopathological examination in diagnosis, classification and prognosis assessment, considering the significantly decreased coagulation function of liver failure patients and liver biopsy examination has certain risk, hence more attention should be paid to indications of liver biopsy in clinic.

1.4 Laboratory Tests of Acute Exacerbation of Chronic Hepatitis B and Severe Hepatitis (Liver Failure)

Xue-Fan Bai

Laboratory tests for liver diseases is the important basis to help and ascertain the clinical diagnosis, and the important reference to evaluate disease severity, make classification, predict outcome and guide therapy. The laboratory tests may reflect the pathological change and the functional status of liver in time, and may provide the objective and detailed data as reference for clinical classification and evaluating therapeutic effects, so that clinical intervention and effective treatment can be performed successfully.

The liver is a complicated organ and the laboratory test items of relevance to severe hepatitis B are many, there are various biochemical items reflecting liver function, including coagulation function, immune and inflammatory cells and genetic markers. In this section only those laboratory tests that are relevant to acute exacerbation of chronic hepatitis B and severe hepatitis B will be described. For nonspecific laboratory tests, the reader is referred to other more general pathology books and literature.

1.4.1 Liver Function

1.4.1.1 Routine Liver Function

Serum Bilirubin

Serum bilirubin is not a sensitive parameter of hepatocellular injury, but a significant increase (commonly ≥ ten times of upper limit of normal value) is usually a specific manifestation of acute exacerbation of chronic hepatitis or liver failure, which is also necessary condition to diagnose severe hepatitis or liver failure. In the course of acute exacerbation of hepatitis both direct and indirect bilirubin rise markedly due to the disturbance of bilirubin metabolism and secretion because the injury and hypofunction of hepatocytes, paracholia, and the rupture of bile capillary and biliary duct.

Test for Enzyme

The main enzymes reflecting liver function are alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (γ-GT, GGT) and cholinesterase (ChE). Enzyme protein content in the liver account for 66.7% of total liver protein. Because the aminopherase content of the liver is 100 times that of blood, in a pathological condition, as long as 1% of the enzyme in the liver is released into blood and keep active, this will be enough to keep the activity of enzyme in the serum increasing at rate of double.

ALT is an enzyme with the highest increasing amplitude and highest positive incidence when acute liver damage is occurring, with activity in the liver 3000 times that of serum. There is a large range of activity in daytime, commonly higher in the afternoon than the morning. Although the activity of ALT is almost coincident with the degree of liver damage, the activity of the enzyme decreases rapidly when liver failure or hepatocyte necrosis becomes widespread, with significantly increasing levels of serum bilirubin, manifesting the disassociation of enzyme and bilirubin. ALT mainly resides in the cytoplasm of the hepatocyte, whilst AST is found more in the mitochondria. When liver cellular necrosis and change of cell membrane permeability appear, there is more release of ALT than AST, but in very severe damage of liver, mitochondria damage is witnessed with elevation of AST and significant elevation of AST/ALT.

ChE in the serum is mainly produced by liver, with its activity and synthesis decreasing when liver damage occurs. Although the change of ChE is less compared

to the aminopherase when liver cell damage occurs, it will drop dramatically when there is severe liver necrosis and liver decompensation, especially in liver encephalopathy.

Plasma Protein Assay

The half life of human serum albumin is 20–26 days. Because the occurrence of low levels of serum albumin is usually a late marker, the albumin level cannot accurately reflect acute liver cell damage, especially during the acute exacerbation of chronic hepatitis B. Serum globulin, especially the γ-globulin, is only elevated in particular chronic hepatitis situations such as decompensated cirrhosis and autoimmune liver disease. The half life of serum prealbumin is only 1.9 days, and its reaction is more rapid and sensitive, which can reflect liver cell damage earlier. Serum prealbumin has special diagnostic value with acute exacerbation of liver especially acute and subacute severe hepatitis.

Lipoid Detection

Total cholesterol is composed by cholesterol ester and free cholesterol in healthy people. When hepatocellular damage appears, its cholesterol esterification noticeably decreases. It has been reported that α lipoprotein decreased significantly in patients with liver failure and this indicates a poor prognosis, while the changes of other lipoprotein and serum triglycerides were not specific for the severe type of patients.

Serum Total Bile Acids

It has been reported that patients with severe liver dysfunction usually have bile acid metabolism abnormalities, indicating that serum total bile acid is a sensitive indicator reflecting functional recovery of liver cell and improvement of pathogenetic condition, just like ALT. Detecting serum total bile acid plays an important role in predicting disease prognosis of severe liver failure and evaluation of therapeutic effect.

1.4.1.2 Other Parameters Correlated with Liver Function

Plasma Kallikrein

Plasma coagulation factor XII (Hageman factor) can produce activating factor XII by surface activation, which can further activate kallikreinogen (also called prekallikrein, PK) to produce kallikrein, Kallikrein then makes prokinin release bradykinin, which cause vasodilatation, increasing capillary permeability with decreasing blood pressure. Because of the short plasma half-life of PK, its plasma content decreases rapidly during liver failure, so PK has important diagnostic value with acute severe type hepatitis and liver failure. It was reported that its content was 92 ± 20% in healthy controls, 30 ± 6% in survivors with decompensatory liver cirrhosis, while only 16 ± 6% in non-survivors. A content level below 23% predicts poor prognosis, with patients usually dying of liver dysfunction within 30–45 days.

Serum Glutathione-S-Transferase (GST)

GST is a protein rich in the liver, the renal tubules and intestine cells in mammals. Its major function is detoxication by combining with multiple metabolistic organics such as bilirubin, bromphenol, cholecystographic agent and epoxide. Because of the high content of GST in the hepatocyte, its small molecular weight and short half-life (only 90 min), it can be released into the blood after hepatocyte necrosis with high concentration. Thus, GST has become a good predictor of observing hepatocyte necrosis especially in patients with acute liver failure and fulminant hepatitis, not only for early diagnosis of hepatocyte necrosis, but also for predicting prognosis.

Other Factors

Other factors such as multiple circular lysosomal enzyme and serum hyaluronate are all significantly elevated during a course of liver failure. These markers can also be used for monitoring and diagnosis of the disease.

1.4.2 Clotting Function

Severe hepatitis and liver failure caused by HBV infection and other pathogenic factors are not obviously different under laboratory examination, although they have different etiological factors, pathogenesis and clinical manifestation. The major liver function, including protein (especial various kinds of coagulation factors) synthesis, metabolism and detoxication, are the first to lose function, and so result in severe hemorrhage and hepatic encephalopathy. Because of the difference on the production of coagulation factors and the steps they involve in, not all coagulation factor detections are suitable for detection of severe type hepatitis and acute exacerbation of hepatitis B.

1.4.2.1 Coagulation Factor Assay

Vitamin K Dependent Factors

Vitamin K dependent factors mainly includes prothrombin (factor II), pre-convert in (factor VII), Christmas factor (factor IX) and fibrin stabilizing factor (factor X). Patients with severe type hepatitis can manifest vitamin K deficiency caused by bile accumulation exterior and interior of liver, ingestion reduction or diarrhea. Factor VII with the shortest half-life (4–6 h) is influenced firstly, which cause prolonged prothrombin time (PT). Factor II is not sensitive on vitamin K deficiency, while factor IX and factor X are moderately sensitive. Factor VII can be considered as a reliable indicator, and it has important clinical value on prediction of prognosis of acute liver failure because of its short half-life and less influence by other factors, such as inflammation, DIC, fibrinolysis, etc. It was reported that when the level of factor VII was below 20% of normal controls, the probability of death increased significantly, with predicting value of 100% sensitivity and 77% specificity.

Human Fibrinogen-like Protein 2/Fibroleukin Prothrombinase

Human fibrinogen-like protein 2/fibroleukin prothrombinase is a mediator of inflammation produced by activated macrophages, which belongs to fibrinogen superfamily and can catalyze and convert prothrombin to activate thrombin directly, thereby starting the clotting process and promoting thrombogenesis. Some studies have demonstrated that the level of human fibrinogen-like protein 2/fibroleukin prothrombinase, if expressed highly and specifically on PBMCs and liver tissue in patients with acute-on-chronic liver failure, can be correlated with disease severity.

Other Clotting Factors

Other clotting factors such as factor VII, XI, XII, I, C protein, plasminogen and platelet count are all decreased in various types of liver diseases, with no special changes on the course of severe-type hepatitis or acute exacerbation. So they are not suitable for evaluation of acute exacerbation of CHB. Some scholars considered that combination detection of antithrombin III (AT III), hepaplastin test (HP) and thrombin time (TT) has important value on early predicting fulminant hepatitis.

1.4.2.2 Clotting Function

Prothrombin Time (PT)

PT is mainly used to detect activity of factor VII, X, II, V and I. It has three measurable methods: one is the prolonged PT, normal PT is 12 to 16 s, with abnormality above 3 s of normal control value; another is prothrombin activity (PTA), which can be calculated by mathematical formula. Normal PTA is 80–120%, usually below 40% in liver failure. The third is international normalized ratio (INR), which can be calculated by certain correction factor: PT in patients/PT in healthy. INR is above 1.2 in abnormality, and it is generally over 1.5 in liver failure. PTA and INR detection values have been included in diagnostic criteria of international and domestic liver failure.

Partial Thromboplastin Time (PTT)

PTT is a screening test of intrinsic coagulation system. PTT can prolong when it is faced with factor VII, IX, XI and XII deficiency or factor I, II, V and X reduction and increment of anticoagulant substances. Because PTT can be prolonged in a variety of liver diseases, demonstrating that PTT is not necessarily specific for the diagnosis of liver failure.

Thrombin Time (TT)

TT tests for the activity of plasma fibrinogen. TT can be prolonged when fibrin degradation product (FDP) is increasing, fibrinogenolysis activity increasing, or fibrinogen (Fib) decreasing, or heparin-like anticoagulant substances occurrence.

Indicators Related with Fibrinolysis

Apart from tissue-type plasminogen activator (tPA) and plasminogen activation inhibitor-1 (PAI-1), other proteins and molecules involved in the course of

fibrinolysis are all synthesized in liver. So plasminogen, α2 antiplasmin and thrombin activation fibrinolysis inhibitor (TAFI) all decreased significantly in severe liver disease such as decompensated cirrhosis. As a result of dysfunction of liver clearance, the tPA level elevates inversely, while with normal or less elevated PAI-1 in patients with cirrhosis, can cause proportion disequilibrium and final hyperfibrinolysis. In patients with acute liver failure, because of large elevation of PAI-1 as acute phase reactive molecule, the activity of fibrinolysis decreases. On the contrary, TAF-1 decreases by almost 50%, which induces elevation of fibrinolysis activity.

Although synthesis of partial coagulation factors and clotting dysfunction usually appear during the course of acute exacerbation of hepatitis B and development of severe-type hepatitis/liver failure, with manifestation of prolonged PT/decreased PTA/elevated INR, some investigators had observed conflicting results recent years. Specifically, although the mean INR was 3.4 ± 1.7 in acute liver injury/acute liver failure patients complicated by hepatic encephalopathy, concentrations of factor V and factor VII also decreased, the mean values of the indicators mentioned above detected by thromboelastography (TEG) were normal, and also five TEG parameters were normal in 63% patients. We think that the reason of normal clotting function detected by TEG in patients with ALI/ALF might be the normal value of platelet count and fibrinogen quantitation. Furthermore, more platelet and factor VII can be produced, and the levels of anticoagulant protein (protein C, protein S and antithrombase) decrease, which also compensates for the defect of the other coagulation factors. In total, clotting parameters such as INR, etc. can be considered as valuable indicators for predicting prognosis, although they cannot be used to reflect hemorrhage severity in patients with ALI/ALF.

1.4.3 Blood Ammonia and Amino Acids

1.4.3.1 Blood Ammonia

Ammonia has been considered as a precipitating factor of hepatic encephalopathy for more than 100 years. During severe liver dysfunction, carbamide synthesis is injured, and brain tissue becomes a major organ of ammonia detoxication. With glutamine synthetase, astrocytes in brain can convert glutamate to glutamine to remove accumulated ammonia in vivo by the amidation of ammonia. Because the synthesis of glutamine consumes energy, the large consumption of ATP can cause energy exhaustion. Excess accumulation of glutamine in astrocytes induced by high blood ammonia can cause increasing osmotic pressure and brain cellular edema. This has been confirmed by MRI, while with the recovery of liver antidotal function after liver transplantation, previous hepatic encephalopathy can be reversed.

In patients with acute liver failure, the risk of cerebral hemorrhage increases rapidly when arterial blood ammonia is above 150 μmol/L. About 55% patients can have acute intracranial hypertension when arterial blood ammonia exceeds 200 μmol/L. Bernal W, et al. in their study which involved 165 patients with acute liver failure, observed that the level of arterial blood ammonia on admission was an

independent risk factor of hepatic encephalopathy and intracranial hypertension, and the sensitivity was 70% for predicting severe hepatic encephalopathy with arterial blood ammonia above 100 μmol/L. Combining with the MELD score can further increase specificity and sensitivity.

The toxicity of ammonia is multiple. The accumulation of ammonia not only influences brain metabolism, injuries brain cellular organelle directly and indirectly, causes disequilibrium of brain internal inhibitory and excitatory neurotransmitter and injuries brain energy metabolism, but also changes a series of gene expressions that are important proteins maintaining brain function. Autoregulation of cerebral blood flow is affected and the blood brain barrier broken down. Latest studies have revealed that ammonia can injury many important functions of neutrophil, such as chemotaxis, phagotrophy and degranulation, and can also stimulate and produce large reactive oxygen species (ROS), cause SIRS, which then further aggravates the toxic effects of brain cells from ammonia resulting in a vicious cycle.

1.4.3.2 Plasma Free Amino Acid Assay

Disequilibrium of Branched-Chain Amino Acid (BCAA)/ Aromatic Amino Acid (ArAA) Ratio

The liver is an important site of amino acids metabolism. Except for BCAA (leucine, isoleucine and valine) metabolism in skeletal muscle, almost all essential amino acid metabolism occurs in liver. Because of this, patients with liver failure or cirrhosis, have large amounts of amino acids accumulate in the blood. Fischer, et al. demonstrated that disequilibrium of plasma amino acids might be reason of encephalopathy, and further indicated that the molar ratio of valine+leucine+isoleucine and phenylalanine +tyrosine (BCAA/AAA) was closely related with the severity of hepatic encephalopathy. The analysis of plasma amino acids in animals with hepatic encephalopathy demonstrated that other concentrations of amino acids increased significantly except the concentration of arginine, which declined. Analysis of brain homogenates from cases of fatal hepatic encephalopathy demonstrated that the concentration of aspartate, arginine and glutamate decreased significantly, and this was closely related with the severity of hepatic encephalopathy. Other amino acids, especially the aromatic amino acids, such as tryptophan, phenylalanine and histidine, increased but with carrying amounts; thus, the concentration of the aromatic amino acids was closely related to the severity of hepatic encephalopathy, implying that these aromatic amino acids may play important roles in the pathogenesis of hepatic encephalopathy, although perhaps not as the primary driver.

Jiang Y, et al. conducted a clinical study which enrolled 22 patients with acute hepatitis, 65 patients with chronic hepatitis, 22 patients with severe hepatitis and 47 cirrhosis patients. They observed the ratio of BCAA and AAA was normal in acute hepatitis, mildly lower in the chronic hepatitis ($P > 0.05$), significantly lower in the severe grade of chronic hepatitis ($P < 0.001$), and the lowest in the patients with severe-type or cirrhosis ($P < 0.05$). As for the Child-Pugh grading, the ratio of BCAA and AAA: C grade <B grade <A grade, with significantly different among the groups ($P < 0.001$ or $P < 0.02$). Of the patients with severe-type hepatitis and

cirrhosis, the ratio of BCAA and AAA in the patients with hepatic encephalopathy was lower than that without hepatic encephalopathy ($P < 0.001$); The ratio of BCAA and AAA was significantly lower in the non-survivors compared with survivors ($P < 0.01$ or $P < 0.005$). They concluded that detection of BCAA/AAA can reflect the degree of liver damage, the lower the ratio was, the worse the liver injury was, and with high risk to develop hepatic encephalopathy; the ratio might also have certain predictive value for prognosis.

Other Free Amino Acid

Elevation of plasma free tryptophan has been correlated with aggravation of hepatic encephalopathy; while in patients with acute liver failure, serum methionine concentrations clearly increase, there is no overlap between acute hepatitis and acute liver failure, and this can be used for differential diagnosis between the two kinds of liver diseases.

1.4.4 Endotoxin and Medium Molecule Substance

1.4.4.1 Endotoxin Assay

Endotoxin is produced by Gram-negative bacteria, and has many biological activities, which can cause endotoxemia, with multiple pathological changes, such as fever, shock, DIC and granulopenia. Critically ill patients even have acute respiratory distress syndrome (ARDS), acute renal failure (ARF) and multiple organ dysfunction syndrome (MODS)/multiple organs failure (MOF).

Wang MR, et al. revealed that the incidence of endotoxemia is 64–100% in severe-type hepatitis patients, 46.5–75.9% in decompensated cirrhosis, 23.5% in compensated cirrhosis, and 36% in acute viral hepatitis.

It has been considered that the main mechanism of endotoxemia in patients with cirrhosis and severe-type hepatitis is: (1) intestine mucosal nutritional disturbance, epithelial atrophy, shedding and ulceration, which cause injury of intestine mucosal barrier; (2) intestine mucosal congestion, edema and increased intestinal wall permeability; (3) alteration of intestinal flora resulting in translocation of bacteria from the intestinal tract and increasing endotoxin production and absorption; (4) low ability of clearance of endotoxin absorbed by intestine or endotoxin into systemic circulation from bypass circuit.

Most studies have demonstrated that endotoxemia and liver injury are interrelated; the liver injury can be aggravated when accompanied by endotoxemia. The detection of endotoxemia is beneficial for predicting clinical course, disease severity and prognosis.

1.4.4.2 Medium Molecule Substance Assay

Medium molecule substance was discovered in patients with uremia accepting hemodialysis, and these molecules have molecular weight of 300–1500 D. Thereafter, it was confirmed to play an important role in hepatic encephalopathy. Medium molecule substance can inhibit Na^+-K^+-ATP enzymatic activity of brain cells, which

result in Na^+ retention and cause cerebral edema. Studies have confirmed that medium molecule substance in the blood increased when acute liver failure patient presented with hepatic encephalopathy, which indicated that an increasing amount of medium molecule substance was closely correlated with hepatic encephalopathy and could be used for predicting clinical severity and prognosis.

As for the property of medium molecule substance, it has not been totally characterized. The clearance of human medium molecule substance is mainly by secretion and filtration from the glomerulus. According to clinic observations, the contents of medium molecule substance can be detected in the plasma when plasma creatinine (Cr) concentration is above 397.8 μmol/L or creatinine clearance rate (CCR) is below 15 mL/min.

1.4.4.3 Blood Lactic Acid and PCT Assay

Lactic acid is an intermediate product of glycometabolism. The concentration of lactic acid can reflect glycometabolism, peripheral circulation, tissue blood-supply and oxygen-supply indirectly. Large studies has demonstrated that total blood lactic acid levels are correlated with disease severity and prognosis. The higher the lactic acid is, the worse the clinical condition is, and also for predicting a poor prognosis. Some studies have revealed that the arterial blood lactic acid has a low predictive value for liver failure caused by hepatitis virus infection, toxin and immunity, but a better predictive value for acute and super-acute liver failure caused by acetaminophen over-dose. However, this is controversial. Schmidt LE, et al. showed that the predictive specificity of lactic acid was not high, even for liver failure caused by acetaminophen. Furthermore, lactic acid cannot be used as an indicator for of liver transplantation, and if used, must be done in association with the current King's College Hospital (KCH) criteria.

Procalcitonin (PCT) is a precursor of human calcitonin, with composed of 116 amino acid residues, 13KD of relative molecular weight, and also has no hormone activity as a glucoprotein. PCT mainly originates from the C cells of the thyroid. During pathologic conditions, multiple tissue and organs can become production areas, such as liver, lung and glandular tissue. The production of PCT can be regulated by bacterial toxin and multiple inflammatory factors, and bacterial toxins are a major stimulating factor inducing production of PCT.

PCT is the best ideal indicator for early diagnosis of systemic infection, and has high specificity. Serum PCT concentration is positively related with the degree of bacterial systemic infection, but can also descend to normal level gradually accompanied with control of bacterial infection and improvement of clinical condition. As for the cirrhosis patients with ascites, detection of serum and ascitic PCT can be of benefit to the clinician for predicting early diagnosis and the curative effect and evaluation of spontaneous bacterial peritonitis (SBP). The PCT levels can decrease significantly in cirrhosis patients with SBP upon effective antibiotics treatment. So, PCT can be considered as a good predictive factor on diagnosis and differential diagnosis of cirrhosis ascites complicated by bacterial infection.

It has been reported that the blood lactic acid and PCT levels were elevated to different levels when facing patients with either acute liver failure or acute

exacerbation of hepatitis B complicated by intestine endotoxemia. A persistently elevated compared to an or not decreasing level of lactic acid and PCT are usually indicative of poor prognosis and therapeutic efficacy. Combined detection of blood lactic acid and PCT does have certain predictive value for acute exacerbation of hepatitis B.

1.4.5 Immunity/Inflammatory Cells and Molecules

1.4.5.1 Regulatory T Cells

CD4+CD25+ regulatory T cells (Treg) can effectively inhibit pathological and physiological immune response, and play an important role in maintaining self tolerance and immune homeostasis. The molecular markers on Tregs include CD4, CD25high, CD127low, CTLA4, glucocorticoid-induced TNF receptor family-related protein (GITR) and forkhead box protein P3 (FoxP3), while all the markers mentioned above are not specific to Tregs.

Regulatory T cells have roles of inhibiting activation of immune competent cell and/or differentiation and amplification. In patients with chronic HBV infection, the abnormality of Treg number and/or function in peripheral blood and liver might cause HBV immune response dysfunction, which may be a major reason of low HBV specific immune ability in the infected body. Stoop et al. first illustrated the relation between Treg and chronic HBV infection. Xu et al. analysed the changes of CD4 + CD25 + Treg in PBMC and liver infiltrating lymphocytes in 16 acute hepatitis B (AHB) patients, 76 chronic hepatitis B (CHB) patients, 29 chronic severe type hepatitis B patients and 42 healthy controls, and demonstrated that Tregs increased significantly in PBMC, FOXP3+ cells and inflammatory cells have also increased in the liver infiltrating lymphocytes. In patients with CHB, peripheral blood Tregs were positively related with serum virus load, while in patients with acute hepatitis B, peripheral blood Tregs experienced process from low to high, and usually are normal in the acute, convalescence, and recovery stages respectively. For the patients their Treg were rejected, the ability of IFN-γ secretion in the PBMCs increased significantly upon antigen stimulation. While the Tregs isolated from the patients with HBV infection could inhibit proliferative response of HBV antigen treated with autologous PBMCs obviously, which might indicate that the peripheral blood and liver in the patients produced HBV antigen specific Tregs. So, Tregs not only play an important role on regulating immune response of HBV infection, but also influence the prognosis of the patients with HBV infection.

1.4.5.2 NK Cells and NKT Cells

Natural killer cell (NK) and natural killer T cells (NKT cells) construct the first line of defense of body to defense pathogen invasion, which account for 13% and 4% respectively in peripheral lymphocytes, while higher with 37% and 26% in the liver. The content of NK cells account for 90% in liver lymphocytes. The accumulation of NK and NKT cells in the liver demonstrates that they have important function, which can directly eliminate virus by killing infecting cells or secreting cytokines.

NK cells originate from marrow lymphoblastoid cells, and do not express specific antigen recognition receptor, but can directly kill tumor and some target cells infected by virus without pre-sensitization by antigen. So, NK cells play important roles in the immunologic process of anti-tumor and early anti-virus infection. Under the condition of virus specific IgG antibody production, NK cells can mediate surface IgG Fc receptor (FcγRIII), identify and kill target cells with complexed IgG specifically by antibody dependent cell mediated cytotoxicity (ADCC). Furthermore, NK cells can also play a role in immunoregulation by secreting cytokines, such as IFN-γ, IL-2 and TNF.

NKT cells are a group of inherent T cells which can recognize lipoid and glycolipid antigen presenting by MHC-I molecules associated with antigen presentation, with CD1d molecule restriction. NKT cells manifest phenotype of both T cells and NK cells (TCR and NK1.1 [NKRP1C], Ly-49). NKT cells have two subtypes: type I NKT cells, also called iNKT cells, and type II NKT cells, also called non-iNKT cells. The important characteristic of iNKT cells is to produce Th1 and Th2 type cytokines rapidly by activation of TCR signal, and also activate multiple other cell types, such as DC, NK cell, B cells, and conventional T cells rapidly by activation of TCR. So, iNKT cells can influence adaptive immunoresponse and series of host defense reaction and pathological course effectively. NKT cells, NK cells and CD8+T cells work together and play important roles in these antiviral processes.

Tian et al. demonstrated that NK cells exist abundantly in normal liver, which account for 33% of lymphocytes within liver and can play important roles on natural immunoresponse of anti-HBV infection. NK cells within the liver can be directly activated by virus or indirectly activated by other cells, such as NKT cells and antigen presenting cells (APC), and exert antiviral effects by their natural cytotoxicity and production of high level of antiviral cytokines. Also they can regulate the function of lymphocytes, such as T cells, B cells, APC, and co-ordinate an adaptive immune response. After the binding of FasL expressed by CTL and NK cells and Fas expressed by liver cell, the intracellular region of Fas protein binds with Fas associated death domain (FADD) protein by its C-terminal death domain (DD), then FADD protein activates Caspase and lead to apoptosis. Malhi H et al. showed that the role of NK cells and NK T cells was a major factor leading to mass death of hepatocyte during the clinical course of viral hepatitis.

1.4.5.3 Dendritic Cells

Dendritic cells (DC) are antigen presenting cells with the most powerful function not only playing an antiviral role by secreting cytokine, such as IL-12 and IFN-α, but also by promoting immunoclearance by T lymphocytes activated via antigen presentation. Furthermore, DC also can regulate the equilibrium of Th1 and Th2. So, the abnormity of number, phenotype and function of DC is bound to influence the outcome of HBV infection.

Some studies illustrated that during liver failure the number of pDCs and mDCs in the liver increased significantly; IFN-α produced by pDCs in the liver

was correlated with the production of IL-12 and IL-10; the ability of IFN-α production by pDCs in the peripheral blood decreased and the degree correlated with disease severity, which indicated that the DCs in the peripheral blood had accumulated in the liver and activated when HBV associated acute-on chronic liver failure occurred. After DC activation, the role of antigen presentation was enhanced, and the immune response activated and even to a peak level. ZH Qian et al. observed that the DC of peripheral blood in patients with chronic severe hepatitis B manifested dysmaturity, dysfunction of cytokine excretion, especially the low secretion of IL-12 which mediated cellular immunity, a high level of secretion of IL-6 and TNF-α which mediated inflammatory reaction, and finally become an important factor of aggravating liver inflammatory reaction leading to severe hepatitis.

1.4.5.4 Th17 Cell

Th17 cell is a new discovered subgroup of CD4+T lymphocytes, with significantly different biological function, cytokine expression and differentiation compared with Th1 and Th2 cells. Th17 cells can mobilize, recruit and activate neutrophil leukocytes by secreting cytokines such as IL-17, IL-6 and TNF-α, in order to mediate inflammatory reactions more effectively.

Zhang et al. detected the Th17 cells in the peripheral blood and liver of chronic hepatitis B (CHB) and HBV associated acute-on-chronic liver failure (ACLF) patients. They observed the level of Th17 cells and its associated cytokines increased in CHB and ACLF patients, and the increasing of Th17 in the peripheral blood and liver is significantly positively associated with HBV DNA load, serum ALT level and histological index of activity. In vitro, IL17 could promote mDCs and monocyte activation, increase its secreting ability of proinflammatory factors, such as IL-6, TNF-α and IL-23, which demonstrated that Th17 might lead to aggravation of liver injury in CHB patients, and Th17 can be considered as one of the immunologic indicators reflecting clinical prognosis of HBV associated ACLF patients.

Wu, et al. observed that the ratio of peripheral Th17 in patients with AHB and severe hepatitis increased significantly compared with general patients with CHB and health volunteers. Th17 cells were significantly positively associated with serum ALT increasing in severe hepatitis, while there was no correlation with HBV DNA quantitation. Furthermore, serum IL-10 levels were significantly negatively associated with the ratio of Th17 cells, which indicated that Th17 cells correlated with the course of HBV and the severity of liver injury. Ye et al. also observed that the ratio of secreting IL-17 and secreting IFN-γ in the liver of the Child-Pugh C patients with HBV infection was significantly higher than that of Child-Pugh B; the numbers of the cells secreting IL-17 in the patients with severe liver injury were more than the cells secreting IFN-γ; the degree of cell infiltration with IL-17 secreting were significantly positively correlated with liver inflammation grading, expressing of IL-8 in the liver and neutrophilic leukocyte infiltration.

1.4.5.5 Related Cytokines

IFN-γ

Chen et al. observed that increasing serum IFN-γ levels was correlated with disease progression of CHB. IFN-γ might be involved in inherent and acquired immunity after HBV infection; IFN-γ can be considered as a potential biomarker for predicting clinical severity of hepatitis B, especially for predicting the tendency of liver failure.

IL-10

IL-10 is a suppressive cytokine which can be produced by Th2 cells, Kupffer cells and liver cells, with major functions of suppressing antigen presentation of macrophage, production of multiple proinflammatory cytokines and Th1 response. Zheng et al. observed that high expression of IL-10 promoted active viral replication in CHB patients, induced excessive immune response. IL-10 can be used to evaluate disease severity and prognosis of severe type B hepatitis. Serum IL-10 levels decreased significantly in severe hepatitis B patients complicated by bacterial infection or overlap with other hepatitis virus infections, which indicated endotoxemia and concurrent viral infection play important roles on promoting occurrence of severe hepatitis B.

TNF-α

TNF-α is mainly produced by active mononuclear macrophages. The concentration of TNF-α is low under normal conditions, with the function of regulating the immune response. Persistent high level of TNF-α can induce liver apoptosis or necrosis. TNF-α has two surface receptors: TNFR1 and TNFR2. Du et al. observed that levels of serum TNF-α, TNFR1 and TNFR2 increased significantly in patients with acute fulminant liver failure compared with subacute fulminant type, acute type patients and health volunteers, and levels of TNFR1 were even higher in non-survivors. Another study showed that expression of TNF-α and TNFR was obviously positively correlated with apoptosis liver cells in patients with fulminant liver failure; TNF-α might lead to widespread liver apoptosis by inducing Fas gene transcription and other signal transduction system. Except for inducing apoptosis, TNF-α can also induce production or release of other cytokines, such as IL-6, IL-8, and so on, to promote intrahepatic inflammatory reaction and aggravate liver cell necrosis by cascade amplified action.

Interferon Induced Protein-10 (IP-10)

IP-10 is a recently discovered CXC chemokine, mainly induced by IFN. IP-10 has strong function of chemiotaxis and activation on T cells expressing specific receptor CXCR3, and also plays important role on lymphocytic accumulating to inflammatory reactive site, which may be an important factor of inflammatory cell migration toward hepatic tissue and causing large area hepatic cell necrosis. Detecting serum IL-10 concentration can indirectly reflect expression of IP-10 in local hepatic inflammatory response. Luo et al. observed that the levels of serum IP-10 in patients

with severe hepatitis B were higher than that of CHB group, indicating the higher degree of liver inflammation and injury, the higher serum IL-10 levels. So, serum levels of IL-10 might reflect the degree of inflammation in hepatitis B patients.

TRAIL-1 and TRAIL2/TRAIL-R1 and TRAIL-R2 System

TRAIL is a new member of TNF α superfamily. A large number of studies indicated TRAIL-R2/DR5 play an important role on apoptosis. The TRAIL induced apoptosis pathway may play an important role in the pathogenesis of severe type hepatitis. TRAIL receptor/ligand system can specifically kill cells infected by virus in HBV infection. Vander Sloot AM et al. observed that affecting HepG2 cell strain by soluble TRAIL produced by cultural monocyte after LPS stimulation can cause apoptosis; higher levels of soluble TRAIL, more obvious apoptosis, which demonstrated that TRAIL induced HepG2 apoptosis was dose dependent. Further studies have shown that serum soluble TRAIL increased significantly in patients with severe type hepatitis and was positively correlated with serum LPS concentration and severity of liver injury, which illustrated that decrease of LPS degradation accompanied with the degree of liver injury can stimulate mononuclear macrophage and DC to express more TRAIL, shedding sTRAIL also increased, cause liver apoptosis and aggravated liver injury after its binding with receptors on liver cells.

IL-22

Interleukin-22 (IL-22) is one of the major cytokines secreted by Th17, and is a member of the IL-10 cytokine family. Th17 is one of the major cells secreting IL-22. Furthermore, active Th1 cells, Th22 cells in CD4+T cell subgroup and inherent immunocytes, such as γδT cells, NKT cells, lymphoid tissue inducer (LTi) cells and NK22 cells also secret IL-22. IL-22 receptor is a heterodimer constituted by IL-22R and IL-10Rβ chain, mainly expressed in some tissues and organs, such as liver cells, epithelial cells of the gastrointestinal tract and skin, while there is no expression in immune cell. It has been shown that IL-22 has characteristics of immunomodulation and immunoprotection in liver and other tissues. IL-22 can induce expression of proinflammatory genes, but also promote liver cell disruption and proliferation, depress liver cell apoptosis. So, Th17 cells and IL-22 might play multiple roles in the immunoresponse to HBV infection.

Zhang et al. detected expression of IL-22 and percentage of peripheral Th17 cell in patients with HBV infection and also detected IL-22 induced antiviral effect, liver inflammation, inflammatory cell chemotaxis and infiltration to liver by using HBV transgenic mice. This group showed that the ratio of peripheral Th17 cell and expression of IL-22 in acute hepatitis B patients were significantly higher than healthy volunteers. IL-22 played important roles on antigen non-specific cells chemotaxis and infiltration, enhancing CTL mediated liver injury. IL-22 cannot effectively inhibit HBV replication in vivo. The degree of liver cell injury induced by HBV transgenic mice, adoptive transferred by spleen cell of HBV immuno mouse, can be improved by neutralizing IL-22. Neutralizing IL-22 can effectively inhibit antigen non-specific inflammatory cells chemotaxis, recruitment and infiltration.

1.4.5.6 Osteopontin

Osteopontin (OPN) is a secretary phosphorylated glucoprotein which contains RGD (arginine, glycine and aspartic acid) protein with an integrin binding region discovered in 1979. It was isolated from bone matrix by Herring in 1983 who found that OPN was a key cytokine which accumulates in immunocytes and initiates Th1 cell immunity, and is involved in multiple pathological process of inflammatory reaction. Some studies demonstrated that levels of peripheral blood OPN were significantly high in patients with severe hepatitis B. It also confirmed that NKT cells can secrete OPN, and OPN also can enlarge activation of NKT cells, to further trigger neutrophil infiltration and activation. Further studies on pathogenesis of OPN in hepatitis demonstrated that the OPN transgenic mice, after been treated with ConA, manifested large necrosis of liver cells and monocytes infiltration, while with only minor liver injury on the controls. So it presumed that OPN might cause immune disequilibrium of Th1 and Th2, induce large hepatonecrosis. In patients with fulminant hepatitis, OPN increased significantly, indicating that OPN can initiate the effect of Th1 cytokine network such as IL-18 and IFN-γ by an autocrine pathway, to further cause macrophage activation and severe hepatonecrosis.

1.4.5.7 Pattern Recognition Receptor

As a specific receptor of endotoxin, toll-like receptor 4 (TLR4) can recognize serum endotoxin, bind with LPS/LBP/CD14, and recruit Myd88, activate IRAK (IL-1R correlated kinase) and MAPKKK (MAPK kinase kinase) family, induce activation of NF-κB, activate target genes transcription, release a series of cytokines, and cause hepatocytes damage. In patients with severe hepatitis, because of intestinal microbial population derangement, bacterial overgrowth and intestinal mucosa congestion, the ability of endotoxin production and absorption increases, which is accompanied by severe hepatonecrosis, immune dysfunction and a decrease in Kupffer cells clearance, which further causes a reduction of exdotoxin and exdotoxin immunocomplex clearance, and finally gives rise to endotoxemia.

Lipopolysaccharide (LPS) is a major component of Gram-negative bacteria, with important physiopathologic function. TLR4 is a key receptor for LPS via signal transduction. Regulating expressing of TLR4 should control LPS related inflammatory reaction. Xu, et al. designed and constructed TLR4 siRNA expression vector, evaluated mouse phagocyte RAW264.7 gene silencing efficiency by transfection, and evaluated therapeutic efficacy of TLR4 gene silencing in the mouse hepatic injury model. It demonstrated that the expression of TLR4 mRNA and protein decreased significantly in RAW264.7 cells; TLR4 siRNA can inhibit obviously the up-regulation of TNF-α and MIP-2 treated with LPS; the activation of p38-MAPK and ERK1/2 by LPS can down-regulate by TLR4 siRNA. Further studies demonstrated pretreatment of TLR4 siRNA can be of benefit to control LPS inflammatory reaction, reduce hepatic lesion of G57BL/6 mouse treated by D-Ga1 N/LPS, and reduce mortality of mice with acute hepatic injury.

Some studies also showed that the TLR4 protein expression on the surface of PBMCs and mRNA were obviously higher in patients with chronic hepatitis B compared with the healthy controls; levels of TLR4 in severe-type patient of CHB were

significantly higher than that of CHB, which indicated TLR4 played important roles during the course of liver injury with HBV infection, and with obvious increasing accompanied with clinical severity. Fan JG, et al. observed the expression of TLR4 increased accompanied with the degree of endotoxemia and the prolong of stimulus duration; LPS can positively up-regulate expression of TLR4 and enlarge biological effect of LPS. So the severe hepatonecrosis and weakening of Kupffer cell deactivation can accelerate the occurrence of endotoxemia, while endotoxin also can regulate liver cell metabolism or influence TLR4 mediated immune reaction to aggravate the clinical situation. The two aspects interact quite closely.

All the studies mentioned above indicated high expression of PBMCs TLR4 in patients with chronic hepatitis, especially with severe-type presentation. Monitoring dynamic changes of TLRs might be benefit for treatment.

1.4.5.8 Inducible Nitric Oxide Synthase (iNOS)

Nitric oxide (NO) is a biological regulatory factor with an extremely short half-life, produced by L-arginine catalyzed by NOS (NO synthetase), with extensive biological functions. It is now believed that NO has potential antiviral inductive activity, and also might be one of the factors mediating liver injury. The studies of mouse model with iNOS defection demonstrated that the mouse model can tolerate CTL induced immunologic injury during the course of inhibiting virus replication because of NO dyssynthesis, and also opposed against injection of fatal anti-Fas antibody, further caused no obvious hepatonecrosis after treated with anti-Fas antibody. In severe-type patients with hepatitis, serum NO levels significantly increase during the early stage, then NO levels decrease significantly with extenuation of inflammatory reaction, liver function recovery in the convalescence phase. These studies revealed that NO is related to the degree of inflammation and clinical severity, and can play an important role in the clinical course of severe-type hepatitis.

Some studies demonstrated that iNOS gene was related closely with viral hepatitis. The expression of iNOS gene was extremely low in normal hepatic cells. Once invasion of hepatic cells by virus appeared, it can induce hepatic cells to activate iNOS gene expression and produce large amounts of NO, not only to kill liver tumor and pathogens such as virus, bacterium, parasites, play broad-spectrum antiviral function, but also injury to adjacent normal liver tissue by cell toxic action, and cause liver cell death. Gong FJ, et al. presumed that pre-S2 protein can down-regulate iNOS transcriptive activity by its trans-regulation, further inducing the decline of NO production and blocking the clearance of HBV in hepatocytes by NO, and finally accomplishing long-term HBV chronic infection. So, expression of iNOS can be considered as an important factor of inflammatory injury.

1.4.5.9 Neutrophil Gelatinase-Associated Lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) was considered as a novel troponin-like biomarker, which was discovered in 1993 by Kjeldsen, et al. who studied neutrophil matrix metalloproteinase 9 (MMP-9) at that time. It was believed that NGAL could be used to monitor acute kidney injury/acute renal failure.

Renal dysfunction is extremely common during the clinical course of severe liver disease such as decompensated cirrhosis and liver failure. Serum Cr levels can increase rapidly when acute renal failure occurs and is easily detected, while it is not easy detected or diagnosed when complicated by chronic kidney injury. Furthermore, other diagnostic methods based upon glomerular filtration rate (GFR) are needed to detect clearance rate, and are not suitable for routine application. The detection of serum Cr is simple, while is not accurate on detection of kidney injury based upon cirrhosis. Gerbes Al, et al. observed 22 cirrhosis patients with ascites whose serum Cr below 1.5 mg/dl, compared the levels of serum NGAL and Cr. The patients was divided into two groups, the GFR was 69 ± 15 mL/min and 29 ± 10 mL/min, and with serum Cr of 0.8 ± 0.2 mg/dL or 1.1 ± 0.3 mg/dL respectively, with no obvious difference. The estimated GFR was 106 ± 34 mL/min and 65 ± 17 mL/min respectively, also with no difference. While in the study, serum NGAL was 50 ± 15 ng/mL and 136 ± 61 ng/mL, demonstrating significant difference ($P < 0.01$). Furthermore, levels of urinary NGAL is similar with the blood NGAL, especially the patients in the group 2, with urinary NGAL above 100 ng/ml. Area under the curve (AUC) analysis also revealed that NGAL was better than serum Cr assay when GFR < 50 mL/min, with AUC = 0.98 (0.96 ~ 1.00) of the former, and 0.79 (0.66 ~ 0.92) of the latter ($P < 0.05$).

1.4.5.10 Macrophage Inflammatory Protein (MIP)

Macrophage inflammatory protein (MIP) is a novel protein first discovered in 1988 by Wolpe, et al. which can be divided into five subtypes: MIP-1, MIP-2, MIP-3, MIP-4 and MIP-5. MIP-2 has a binding site for heparin, which can interact with heparin dextran sulphate in endothelial extracellular matrix, and enhance adhesion of leucocytes and vascular endothelial cells. MIP-2 is involved in the whole process of inflammation by chemical chemotaxis and activation of inflammatory cells, with neutrophils as specific target cells. MIP-2 can also activate neutrophils specifically and has been considered as an important proinflammatory cytokine during the early stage of inflammation.

Chen Z, et al. observed that the levels of MIP-2 in severe-type patients with hepatitis B was significantly higher than other groups. Persistent high expression of MIP-2 can be used to predict acute exacerbation of hepatitis B and clinical severity of patients with hepatitis B.

1.4.5.11 Thymosinβ4

Thymosinβ4 (Tβ4) is one of the important actin regulatory molecules in human body. It can bind with globular actin (G-actin), inhibit production of fibrous actin (F-actin), reduce formation of microthrombi and microcirculation dysfunction in patients with liver failure, and inhibit occurrence and development of MOF. Tβ4 can also reduce the levels of free radicals, slow lipid peroxidation and inhibit production of proinflammatory factors. Patients with liver failure usually manifest a large amount of mononuclear macrophage infiltration, secretion, releasing proinflammatory factors and aggravate systemic inflammatory reaction. Liu Y, et al. observed that the levels of serum Tβ4 decreased significantly in patients with liver failure

compared with the cirrhosis group, CHB group and healthy controls. Dynamic changes of serum Tβ4 can be considered as a novel predictive indicator for prognosis in patients with liver failure.

1.4.5.12 Tim-3

T cell immunoglobulin mucin 3 (Tim-3) is a surface molecule correlated with function of T cells, which was discovered in 2002. The studies on mouse model revealed that Tim-3 can be expressed on the surface of Th1 selectively as a negative regulatory molecule; the interaction of Tim-3 and its ligand plays a down regulatory role on Th1 cellular mediated immune response. Sabatos CA, et al. observed that the levels of peripheral Tim-3 in CHB patients increased significantly, and it indicated the expression of Tim-3 can be induced by regulating Treg whose activity was modulated during the chronic infection period in patients with CHB, and then inhibit specific Th1 cell responses.

Zhou XQ, et al. observed that the levels of Tim-3 was significantly higher than other groups in severe-type patients with hepatitis B, which can be used to predict and monitor the clinical severity of hepatitis B patients. With the progression of CHB, serum iNOS and NGAL all increased, with the highest levels in severe grade of CHB, while decreased inversely in the serum of the severe-type patients with hepatitis B, which can predict acute exacerbation of chronic hepatitis B. It was reported that the crosspoint of persistent elevation of Tim3 and MIP2, and declining of iNOS and NGAL, demonstrated important clinical significance on predicting acute exacerbation of CHB.

1.4.6 Related Genetic Symbols

1.4.6.1 Sex Hormone Signal Pathway and Acute Exacerbation of CHB

Recent studies have demonstrated that genetic variation of hormone receptor gene was significantly different between CHB patients with acute exacerbation and HBV carriers, which could explain the sexual difference on different clinical course of hepatitis B.

Clinical studies further demonstrated that male patients with HBV infection had higher incidence and ratio of chronicity; male patients with HBV infection had higher activity frequency, which indicated the sex hormone and its receptor might influence the process of HBV infection by regulating host immuno response and level of viral replication. It is now believed that immunoresponse of viral clearance might down-regulate male sex hormones and up-regulate female sex hormones. Furthermore, steroid hormones might regulate replication of HBV mediated by androgen receptors. It has been proved that androgenic hormone can inhibit cellular and humoral immune reaction; estrogenic hormone can inhibit cellular immunoresponse and enlarge humoral immunoresponse inversely; progestogen can promote conversion from cellular immunity to humoral immuno reactivity.

Several studies have reported the action of estrogen receptor (ESRs) in the diseases of multifactorial inheritance, and its roles have also been observed by

geneticists and virologists. Large number of studies have demonstrated that ESR2 played important roles on regulation in patients with persistent HBV infection, and ESRs genes were correlated with host hereditary susceptibility. Because of low expression of ESRs in HBV carriers, the reaction of immune system to sex hormone was insufficient, which make clearance of HBV difficult and can lead to HBV persistent infection. Human ESRs are divided into two types: estrogen receptor A (ESR1) and estrogen receptor B (ESR2). All the genetic variations can cause degression of estrogenic function, which may further cause different kinds of host hereditary susceptibility after HBV infection. Of them, ESR1 gene can mutate, with tendency of sex hormonal resistance. Sex hormone mainly plays the role of binding ESR1, and ESR1 gene is the true minor gene with host hereditary susceptibility after HBV infection. Deng GH, et al. observed the relationship between ESR1 gene polymorphism and chronic persistent HBV infection in a large sample study. They found that the susceptibility increased significantly compared with the individual of ESR1 29T/T genotype and the individual containing at least one 29C isoloci site ($P < 0.001$). Linkage disequilibrium plotting analysis indicated that T29C polymorphism included a linkage disequilibrium area sited from ESR1 promoter to intron 3, which indicated that the ESR1 T29C convention originated from ESR1 itself, and ESR1 gene polymorphism in the Chinese population was positively related with HBV persistent infection.

The negative feedback regulation of hypothalamus-pituitary gland-genital gland axis can be performed by serum testosterone (T) and estradiol (E2) through central nervous system feedback, and control releasing of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Some studies demonstrated that the levels of serum T and E2 decreased in male patients with hepatitis B cirrhosis, and the levels of LH and FSH increased correspondingly, which indicated liver injury might be an important reason of sex and hypophyseal hormone abnormality. Until now, the studies on changes of sex and hypophyseal hormone in acute exacerbation of hepatitis B and severe-type hepatitis are still limited, while it is certain that precise detection of sex hormone and correlated hypophyseal hormone can reflect the severity of liver injury to a certain degree, and can be considered as a useful indicator evaluating the clinical course of hepatitis B, cirrhosis and its complication, hepatocellular carcinoma.

1.4.6.2 Antiviral Immunoresponse Genes and Acute Exacerbation of Hepatitis B

It is now believed that the priming or control point of severe-type hepatitis occurrence and development is influenced by two aspects: virus (such as viral variation, viral protein and viral genotype) and host (such as cell immunity, cytokines and apoptosis), and of them, viral replication is the necessary condition and cause of severe-type hepatitis. Some studies demonstrated that viral replication was relative vigorous during the early stage of severe-type hepatitis, which indicated viral factor occupy a central position in the occurrence and development of acute exacerbation of hepatitis B. The changes of host transcription factors and HBV genetic variation

all possibly cause the changes of HBV transcription and replication, resulting in acute exacerbation of hepatitis B.

Hepatocyte nuclear factor 4 (HNF4) is a protein with the function of genetic transcription and modulation, and is mainly found in liver. Long CE, et al. observed that the HNF4 binding sites, located in Enh I/Xp and EnhII/C promoter, is an important factor regulating C promoter activity and 3.5 kb mRNA transcription, and has an important role on HBV replication and expression with high tissue specificity. Previous studies demonstrated the expression of HNF4 increased in patients with CHB, and the level of HNF4 is positively correlated with HBV replication. Furthermore, from a study on liver biopsy specimens using gene array in patients with HBV infection, Honda M, et al. observed HBV infection can cause more gene transcription of HNF4, RXR/PPAR and C/EBP, and possibly related with liver injury. So, the interaction of HNF4 and HBV genetic transcription and control might play an important role on the pathogenesis of severe-type hepatitis B.

Deng et al. studied CXCL10 SNP variation on Th1 immune response pathway, and observed that the polymorphism site G-201A existed in CXCL10 gene promoter region was significantly related with disease progression in male HBV carriers. An EMSA study revealed that the G-201A site can change its binding ability with nucleoprotein; and reporter gene assays also revealed that this site can influence the transcriptional activity of CXCL10 gene promoter region; mRNA real time quantitative experiments further revealed that the expression of susceptible allelotype -201G in PBMCs was significantly higher than allelotype -201A; ELISA and immunohistochemical assays revealed a higher expression of CXCL10 protein in plasma and liver tissue of patients with CHB that progressed than that of non-progressive patients. All the studies mentioned above demonstrated that G-201A is a new functional polymorphism site, CXCL10 protein involved in the process of inflammation and necrosis in liver tissue of patients with hepatitis B, and influence the progression of CHB.

Furthermore, Yan Z, et al. reported that mutation of IFN-γ gene can influence expression of IFN-γ, which is a predisposing factor of chronicity of HBV infection; fulminant hepatitis patients with poor prognosis had high frequency of occurrence of -1031C, -863A and TNF-βB2 allele in TNF-α gene promoter region; SNP in IL-10 gene promoter region was related with progression of chronic HBV infection; the frequency of haplotype expressed by down-regulated IL-10 in the IL-10 gene promoter region in patients with fulminant hepatitis was higher than the controls, while the frequency of haplotype gene occurrence by up-regulated IL-10 expression was even lower. Yan et al. also revealed the relationship between polymorphism of IL-10 gene promoter in Th2 immuno response pathway and chronic severe-type hepatitis, which provided new evidence on natural selection theory of IL-10 promoter and SIRS pathophysiology theory of acute liver failure.

In a study of human fibrinogen-like protein 2 (hfg12) gene regulation mechanism and network, Han et al. observed that HBc protein and HBx protein all had the function of hfg12 activation, while HBs protein cannot activate the gene. A series of promoter deletion experiments demonstrated that the regulation

sequence of activating hfg12 gene existed between the sits of hfgl2 gene promoter −712 ~ −568, transcription factor c-Ets-2 can bind with cis-acting element on hfg12 gene promoter, then activate the expression of the gene under the action of that viral protein; the phosphorylation level of P-JNK and P-ERK was enhanced in patients with severe hepatitis B than healthy controls, which indicated JNK and ERK signal pathways were activated. Some studies in vitro revealed that JNK and ERK signal pathways were activated respectively under the effect of viral protein HBx and HBc, and c-Ets-2 expression and transposition were correlated with the activity of JNK and ERK. All the studies mentioned above indicated that viral protein HBc and HBx can activate JNK pathway and ERK pathway respectively, further activate transcription factor c-Ets-2, shift into the nuclear, and finally up-regulate the expression of hfg12 gene by binding with cis-acting element of hfg12 gene promoter. TNF-α is an important proinflammatory factor induced during liver failure. Mfg12/prothrombinase plays an important role on mouse fulminant hepatitis and acute on chronic liver failure. Gao et al. constructed eukaryotic expression vector of mfg12 gene and TNFR1 gene, green color fluorescence fusion protein and its shRNA interfere plasmid. It was more effective to improve the survival rate of mouse with severe type hepatitis using combination of fg12 and TNFR1 genes compared with single gene intervention, and improve serology and pathological changes of liver, which indicated that fg12 and TNFR1 genes might produce synergistic effect during the progression of severe-type hepatitis.

HBV infection is a necessary condition and cause of occurrence and progress of severe-type hepatitis, while HBV gene transcription and replication are a key step of the HBV life cycle. Beginning from the new point of genetic transcription, further exploring the effect and mechanism of acute exacerbation of hepatitis B on HBV genetic transcription and regulation, will provide important clue of host transcription factor, which can be useful to predict and monitor the occurrence, development of severe-type hepatitis, and to provide new targets for effective methods of treatment of severe-type hepatitis.

1.4.6.3 Genome-Wide Association Study and Acute Exacerbation of Hepatitis B

Genome-wide association study (GWAS) has been available since 2005, and have become the main aspect of complicated genome association study. The strategy does not need to select candidate genes, but to make genome-wide associations and analysis by selecting directly ten thousand single nucleotide polymorphism (SNP) sites and copy number variation (SNV) sites over the total genome. Compared with the strategy of a candidate gene, GWAS was a significant improvement in statistical efficacy, and also avoided the bias of group stratification and randomness of gene selection.

In recent years, some studies on the aspects of viral hepatitis and HBV related hepatocyte carcinoma has gained great breakthrough using these approaches. Several independent and large sample GWAS in USA, Europe and Japan demonstrated that only rs1297980 site near the IL-28B gene had relationship with curative

effect of interferon in the study on genetic correlation of IFN response in patients with hepatitis C (with *P* value of 10^{-25}).

Guo et al. observed that gene variation of HLP-DP gene can play great influence on clinical severity of persistent chronic HBV carriers. Karlsen et al. also reported the relation between HLA-II gene and HBV infection. He FC and Zhou GQ made GWAS of HBV related hepatoma, and they discovered a related site 1p36.22, where one of the three genes (KIF1B, UBE4B, PGD) might be predisposing gene of HBV related hepatoma.

The studies on genetic predisposition of severe-type hepatitis B are relatively less. Only a minority of studies have examined a candidate gene—disease associated study strategy, and their study populations were all from Asia. Recently, under the support of the national 973 program and key state science and technology project of infectious disease, Chinese experts have taken Affymetrix SNP 6.0 array (including 900,000 SNP sites and 900,000 CNV sites in the range of human total genome-wide) to make GWAS in 1600 severe-type patients with hepatitis B and asymptomatic carriers. The project is now being analyzed, expecting to explore common genetic variance sites influencing severe-type hepatitis B under the point of host total genome.

1.4.6.4 Epigenetics and Acute Exacerbation of Hepatitis B

Epigenetics mainly studies the change of herediable gene expression with no change of DNA sequence. Currently, epigenetic mutation mainly includes DNA methylation, microRNA (miRNA) and chromatin remodeling.

miRNA

miRNA is a highly conserved non-encoding RNA, which can regulate gene expression on post-transcriptional level. Large studies demonstrated that miRNA has differential expression during the course of chronic injury, especially chronic liver injury.

Gao S, et al. constructed miRNA expression plasmid of hFas and hTNFR1 genes, and made cellular experiments in vitro, which demonstrated miRNA expression plasmid of hFas and hTNFR1 genes can inhibit corresponding genes specifically, and have the potential value of clinical application on treatment of severe-type hepatitis induced by virus.

Several functional studies also demonstrated that miRNA participated and regulated the expression of multiple inflammatory signal factors. It was found that miR-150 and miR-223 might be linked with liver inflammatory activity of viral hepatitis. miR-150 mainly participated in regulating maturation of B lymphocytes, and also regulate the differentiation of Th1 and Th2 cells. miR-223 located in human x chromosome, and highly expressed specifically in granulocyte series cells. Johnnidis, et al. observed the granular leukocytes increased twice in miR-223 gene knock out mouse, and these leukocytes were more sensitive when treated with antigenic stimulation outside (especial fungi); compared with miR-223 gene non-knock out mouse, the lung inflammation of miR-223 gene knock out mouse was more obvious, and the tissue injury induced by exdotoxin stimulation even more serious.

The latest studies have discovered seven susceptible molecules correlated with acute exacerbation of hepatitis B: miR-478a (down-regulation in severe-type twins patients with hepatitis B), miR-7a (up-regulation in severe-type patients with hepatitis B), miR-16 (up-regulation in severe-type patients with hepatitis B), miR-122 (providing early warning signal on acute exacerbation of hepatitis B and disease severity), miR-1187 (has obvious decline tendency with the development of liver failure, and can be considered as biomarker for predicting clinical severity and acute exacerbation of hepatitis B), miR-155 (its expression decreases gradually accompanied with viral clearance, can be considered a biomarker of turnover of acute exacerbation of hepatitis B, and provide early warning signal on acute exacerbation of hepatitis B and treatment), miR-197 (down-regulation has predictive value to liver inflammation).

It has been finally shown that detection of specific miRNA correlates with liver inflammation, by using miRNA array technique in severe-type patients, can provide novel target on early prediction of severe-type hepatitis.

DNA Methylation

Qi ZX, et al. observed the difference of Th1 and Th2 cells chromatinization status in IL-10 gene promoter region, which interfered with the binding of promoter and transcription factors, and influenced gene transcription of IL-10. The distribution of methylation in patients with liver failure showed obvious differences between CHB patients and healthy controls; concentrations of serum IL-10 were negatively related with promoter methylation status, which indicated the methylation of IL-10 gene promoter region might involve in the pathogenesis of liver failure, and it is also a reason of IL-10 change. Furthermore, some studies revealed that methylation of glutathione-S-transferase P1 promoter region can promote acute exacerbation of hepatitis B.

1.4.7 Other Biomarkers Correlated with Acute Exacerbation of Hepatitis B

1.4.7.1 Activation of Toll-like Receptor Signal Pathway

Toll-like receptors (TLRs) mediated signal transduction pathway includes two forms: myeloid differentiation factor-88 (MyD88) dependent form and MyD88 independent form. TLR7, TLR8 and TLR9 mediated signal pathway belongs to MyD88 dependent form pathway; TLR3 mediated signal pathway belongs to MyD88 independent form or TRIF independent form signal pathway. In the MyD88 independent form pathway, TLRs recognized ligand, then activate NF-κB and MAPK, induce release of multiple cytokines, up-regulate costimulatory molecules such as CD80, CD86, et al., then finally activate specific immune system.

TLRs and TLR4 in particular, play important roles on the mechanism of acute exacerbation of hepatitis B, which can better predict tendency of the disease, and can be considered as indicators for evaluating prognosis of severe-type hepatitis B.

TLR4 is a key receptor in endotoxin transmembrane signal transduction; it is also a rate limiting factor in LPS transmembrane signal transduction of mononuclear cell and macrophage. Some studies demonstrated that the expression of TLR mRNA in severe-type patients with viral hepatitis was higher than the healthy controls; and it is higher during the aggressive stage than the critical stage in the non-survivors and it is lower during improvement than the critical stage in the survivors. Furthermore, expression of TLR4 mRNA is positively correlated with the levels of endotoxin. Deng M, et al. observed that the severe-type patients with viral hepatitis had tendency to endotoxemia, with endotoxin binding with TLR4, then transmembrane signal activation of NF-κB through intracellular structure of the TLR4, further induced release of inflammatory factors, such as TNF-α and IL-1, and produce secondary intrahepatic microcirculation disturbances.

TLR2 is a pattern recognition receptor with extensive pathogen recognition abilities. TLR2 can recognize cell walls from multiple bacteria such as Gram-positive bacteria, Gram-negative bacteria, fungi, spirochaetes, Mycobacterium tuberculosis and Mycoplasma, and play important roles on acute and chronic infection. Yan CG, et al. observed the expression of TLR2 of mouse with fulminant liver failure induced by D-Gal/LPS. They observed the high and persistent expression of TLR2 mRNA, and accompanied with high expression of TNF-α, which indicated that TLR2 can up-regulate expression of downstream inflammatory response genes and release of cytokines, to involve in acute liver failure induced by D-Gal/LPS.

1.4.7.2 Changes of Metabolomics During the Course of Acute Exacerbation of Hepatitis B

Metabolomics is a new technology, which studies various changes of endogenous metabolic products and biologic conditions in humans by observing biosystem changes qualitatively and quantitatively under various stimulations and changes of internal and external environment. The study object is the micromolecule compound with relative molecular mass < 1000 in the metabolic network. At present, the major techniques include nuclear magnetic resonance spectrum (NMR), gas chromatogram (GC), liquid chromatography (LC), capillary electrophoresis (CE), mass spectroscopy (MS), infrared spectrum and ultraviolet spectra. Of them, NMR, GC-MC and LC-MC are used more extensively.

Yang et al. investigated the serological profile in acute hepatitis B patients with sudden aggravation of liver function by HPLC-MS and PLS analytical techniques. They observed the changes in individual atoms in lysophosphatidyl choline (LPC) and GCDCA, which can predict early disease aggravation. Feng B, et al. analysed the metabolic product in serum of rats with fulminant liver failure by GC-MC and PCA software, and observed high content of 5-hydroxyindole acetic acid, glucose, β-hydroxybutyric acid and phosphate, which indicated the changes of these substances might become potential biomarkers of fulminant liver failure. Yu, et al. set up a research platform based upon liver failure metabolomics by using gas chromatographic mass spectrometry (GCMS) and super-high performance liquid chromatogram mass spectrometry technology, constructed a model for evaluating clinical severity of liver failure, and constructed serum specific metabolism

spectrum by partial least squares discriminant analysis, which can be used to predict the prognosis, with sensitivity of 91.3%. Mao et al. also derived similar results with a high positive diagnostic rate of 93.62% for liver failure, and also found that the changes of climax concentration of plasma citrate had potential diagnostic significance.

Li LJ, et al. pointed out the low level of lysolecithin, high level of fatty amide and nonelevation of bile acid by using metabolomics were risk factors for predicting poor prognosis in liver failure patients with pretreatment and post-treatment by artificial liver support systems. By observing the trend changes of serum metabolic spectrum in patients with pretreatment and post-treatment with an artificial liver, we can predict the prognosis and assess curative effect of artificial liver.

1.4.8 Detection of Plasma Electrolytes and Acid Base

1.4.8.1 Plasma Electrolytes

Electrolyte disturbances were usually complicated in patients with liver failure because the patients typically have low renal blood flow and glomerular filtration rate (GFR). A majority of patients manifest hypokalemic, hyponatremia, hypomagnesiumemia and hypocalcemia. The occurrence of hyponatremia and its severity are positively related with prognosis in patient with liver failure. Severe hyponatremia can cause acute low sodium syndrome and encephalopathy; and hyponatremia can also cause renal injury and promote or aggravate development of hepatic encephalopathy and hepatorenal syndrome (HRS), and increase mortality. Some studies indicated that the mortality of the patients with severe hyponatremia (<115 mmol/L) is extremely high and up to 93.88%.

In patients with acute liver failure, hypochloraemia can be caused by insufficient intake of chlorine, vomiting, large excretion of aldosterone and acidosis, while water intake insufficiency, renal failure, renal tubular acidosis and potassium chloride overdose can cause hyperchloraemia inversely. Furthermore, hypophosphatemia is more readily detected than hypocalcemia in most patients.

1.4.8.2 Acid-Base Equilibrium Examination (Blood Gas Analysis)

Acid base disorders (ABD) are very common in patients with acute liver failure, especially alkalemia. Li XM, et al. presumed that arterial carbon dioxide pressure depression and respiratory alkalosis are frequent manifestations of acid base disorders. ABD is not related with basic pathological changes of severe-type hepatitis, whilst blood pH value is an important factor influencing the survival outcome of severe-type patients with hepatitis.

In the latest years, some scholars think that pre-estimation of compensatory formula (PCF) for ABD has practical value on differential diagnosis of partial simple and duality ABD. Partial patients with acute liver failure complicated by ABD can be diagnosed by anion gap (AG), potential HCO_3^{-} and Cl^{-}. Elevation of AG is a reliable indicator of appearing potential metabolic acidosis, and is common in

patients with hepatic encephalopathy, lactic acidosis caused by hypoxemia. During hepatorenal syndrome since reduction of acidum excretion of kidney ketoacidosis may appear. Potential HCO_3^{-} is the HCO_3^{-} value exclusive of the masking HCO_3^{-} by high AG metabolic acidosis, which can raise the existence of AG metabolic acidosis and metabolic alkalosis with triplicity ABD. A mixed metabolic acidosis can be discerned by combination of AG and blood CI.

Series of primary ABD can overlap, even to develop duplex and triplication ABD. For example, if the respiratory alkalosis prolongs, accompanied with increasing of potassium excretion by renal, and with iatrogenic factors to develop metabolic alkalosis, and further develop multiple complications combined with metabolic acidosis, which finally develop triplicated ABD. Triplication ABD is usually the end stage of acute liver failure, with worst prognosis. So, prevention and active treatment of ABD is an important parameter for rescuing patients with acute liver failure.

1.4.9 Micro-Invasive Examinations

1.4.9.1 Liver Biopsy

Liver biopsy is a good method to acquire pathologic changes of liver in multiple liver diseases including liver failure, which have important value on directly evaluating the severity of liver damage.

Liver biopsy is generally divided into percutaneous biopsy, transvenous biopsy, surgical/laparoscopic biopsy and plugged biopsy. Of them, percutaneous biopsy is safe and simple, without special instrument or equipment, and being used extensively. Transvenous biopsy is more suitable to the patients with large amount of ascites, clotting dysfunction, diminution of liver volume and increasing of liver hardness because of cirrhosis, difficulty to determine hypochondria because of obvious obesity, and with high hepatic vein pressure.

The contraindication of liver biopsy are as the follows: (1) excessively prolonged PT, excessive declining of PTA, which might cause haemostasis difficult even mild injury; (2) obvious cavity bleeding or large area of petechia; (3) patients with unconsciousness, who might not be able to fit the operation; (4) medium dose ascites, especially patients complicated by abdominal infection; (5) patients with obvious pleural effusion and severe heart and lung disease.

It has been proved that liver biopsy is safe to a great extent under careful preparation, attentive operation, even in the liver failure patients with obvious clotting dysfunction. What we need to point out is that although liver biopsy has important value on recognizing liver tissue degeneration, necrosis and degree of inflammatory infiltration, the pathologic change of the biopsy tissue might not be able to reflect entire pathologic change of the liver because of the limited liver tissue obtained. So, it is very important for clinicians to collect and combine various forms of clinical data, laboratory and pathogenetic results from the patients, for getting an accurate judgment.

1.4.10 Measurement of Intracranial Pressure

The incidence of cerebral edema in severe-type patients is high, with ratio of 50 to 80%, and a quarter of them can appear cerebral hernia with poor prognosis. So, measurement of intracranial pressure plays important role on diagnosis and treatment of cerebral edema.

Traditional lumbar puncture manometry can measure intracranial pressure only one time; it cannot be observed that the changes of intracranial pressure dynamically and exactly. On the other hand, lumbar puncture on patients with acute intracranial hypertension might cause or aggravate cerebral hernia. Furthermore, under the condition of cerebral hernia, cranial cavity and spinal cord cavity can not intercross, so the pressure detected by lumbar puncture cannot represent actual intracal pressure. So, persistent detection of intracranial pressure retrieves shortage of lumbar puncture, and cerebral ventricle catheterization is still an extensively used method, which is considered as gold standard of intracranial pressure monitoring by clinicians.

Intracranial hypertension is defined as persistent elevation of intracranial pressure with levels above 15 mmHg. According to the difference of intracranial pressure, intracranial pressure can be divided into four stages: (1) normal: 5 ~ 15 mmHg; (2) mild: 15 ~ 20 mmHg; (3) moderate: 20 ~ 40 mmHg; (4) severe: above 40 mmHg. If intracranial pressure is close to mean arterial pressure (MAP), which illustrates almost no brain perfusion, with nearly close to brain death. Currently, 20 mmHg is defined as the critical value of applying treatment of anti-intracranial hypertension.

Complications of intracranial pressure measurement include infection, intracranial hemorrhage, iatrogenic acute intracranial hypertension and brain parenchyma injury.

1.4.11 Summary

Although laboratory parameters can reflect pathological changes and liver functions during the clinical course of severe-type hepatitis and acute exacerbation of hepatitis B sensitively and in time, and can provide detailed reference data for clinical typing, grading and curative effect evaluation, in total, the extensive and large numbers of indicators listed above are still under study and experimental evaluation, and their availability and utility need to be checked by more clinical practice. Until now, laboratory parameters have been extensively used and applied clinically but are still limited. The laboratory parameters conventionally tested in the majority of hospitals are mainly PTA, INR, serum bilirubin, ALT/AST, pre-protein and blood ammonia. Furthermore, some studies also reveal that serum AFP, serum sodium and phosphate are closely related with prognosis of liver failure.

Although some indicators such as arterial blood lactic acid, PCT, NAGL and iNOS are closely related with severe-type hepatitis or liver failure, they also score as abnormal in other severe diseases or conditions such as septic shock and MODS, so these parameters are not specific for severe-type hepatitis B and liver failure. It is

the same on the indicators related with immunity or inflammatory cell or factors, with high sensitivity, while without specificity. Other indictors related with heredity, metabolomics, genomics, proteomics and glycomics are still in research, or needing expensive instruments, and still difficult to the routine clinical practice in the short term.

What we need to point out is that the various kinds of prognosis scoring systems and mathematical models constructed and tested in the latest years, such as Royal School of Medicine Standard in United Kingdom, model for end-stage liver disease (MELD) and Child-Pugh scores, have higher accuracy and utility on predicting prognosis of severe-type hepatitis and liver failure, although some of them still need additional supplement and improvement in the future.

Because of diversity and individualization for particular causative factors, clinical types, clinical course, complications and clinical treatments, until now, there are still many challenges reflecting prognosis of liver failure. It is believed that more and more novel biomarkers will come into the clinic in the near future, and with development of studies of mechanism and clinical characteristics on acute exacerbation of hepatitis B, therapeutic efficacy of severe-type hepatitis and liver failure will be improved.

1.5 Virological Detection for Acute Exacerbation of Chronic Hepatitis B and Severe Hepatitis (Liver Failure)

Xiao-Jing Wang

Pathogenesis of AECHB is generally considered to include viral factors (HBV genotype, viral replication and viral mutation, etc.), host factors (host genetic characteristics, immune injury, cell apoptosis and necrosis) and interaction between these two aspects.

1.5.1 HBV Genotype and AECHB

1.5.1.1 Global Distribution of HBV Genotype

Currently, there are at least ten different HBV genotypes and several subtypes distributed all over the world (see Table 1.6) based on the differences of whole genome sequences >8% for the major genotypes or 4–8% at the sub-genotype level.

Genotype A is mainly distributed in northern Europe (A2), west Africa (A3), and sub-Saharan Africa (A1). In Asia, genotype B and C are most common. Genotype B have six subtypes (B1–B6), in which, B1 was found in Japan, B2 ~ B5 were found in east Asia, and B6 was found mainly in the arctic regions, such as Alaska, northern Canada and Greenland. Genotypes B2-B5 are also regarded as recombinants with genotype C. Genotype C, including five subtypes (C1 ~ C5), is mainly distributed in East Asia and Southeast Asia. Genotype D, including subtypes D1 ~ D5, prevails in Africa, Europe, Mediterranean and India. Genotype E is only found in West Africa.

Table 1.6 Global distribution of HBV genotype and subtype

Genotype	Distribution
A	North America, West Europe
B, C	Asia (China, Japan)
D	South Europe, Africa, India
E	West Africa
F	Central America, South America, Alaska
G	America, France, Germany
H	Central America
I	Vietnam, Laos
J	Japan

Genotype F, having four subtypes, is found in central and South America. Genotype G has been reported popular in France, Germany and the United States. Genotype H is found in Central America [2–4]. Genotype I was recently newly found in Vietnam and Laos, but this new genotype is under controversial [5]. The newly found genotype J in Japan has a close relationship with the orangutan's genotype and human genotype C. Population migration, promotion of antiviral therapy and host immune selection pressures result in increased risk of HBV gene recombination or mutation [6]. With the development of genetic testing methods, even more new genotypes could still discovered.

1.5.1.2 HBV Genotype and Severe Hepatitis B

Due to the distribution differences of HBV genotypes, the study on the relationship between HBV genotypes and severe hepatitis B is quite limited. Comparisons have only been conducted in a few genotypes. A study from United States on patients with HBV related acute liver failure suggested that outcomes of these patients were not associated with genotypes [7]. A multicenter study from Japan involving 301 patients with acute HBV infection has compared 5 genotypes (Ae [A2],Ba [B2–5],Bj [B1],Ce [C2],Cs [C1],DandG). Multi-factor regression analysis indicated that subtype Bj(B1) is one of the independent predictors for fulminant hepatitis. Subtype Ae(A2) is more related to HBV persistence but not self-limiting hepatitis B [8]. Another multicenter study from Japan showed that patients with genotype C accounted for 80 and 69% in patients with acute-on-chronic liver failure and acute liver failure, respectively. These rates are much higher than that in patients with chronic infection, suggesting that patients with genotype C are more likely to suffer from fulminant liver failure [9]. A study from China included 26 HBV carriers, 61 patients with chronic hepatitis B, 20 patients with ACLF, 12 HBV related liver cirrhosis and 7 patients with HBV related HCC. Data showed that genotype B (38.1%) and C (54.8%) were the main genotypes in these patients. Compared with genotype B, genotype C was seen more frequently in those with severe liver diseases, was accompanied with high levels of HBV replication, indicating that genotype C is associated with high HBV replication and severe liver disease [10]. However, results from another study showed no difference in genotype composition among patients with chronic hepatitis B and those with chronic severe hepatitis [11]. In a study

involving 487 HBV-infected pediatric patients, in which 217 patients had been treated with nucleos(t)ide analogues, genotype C2 and B2 were found to be the most prevalent subtypes (73.7 and 21.1%). Compared with genotype B2, genotype C2 is more likely to cause severe hepatitis in HBeAg positive pediatric patients [12]. The association between various genotypes and the pathogenesis of severe hepatitis needs further studies.

1.5.2 HBV Gene Mutation and Severe Hepatitis B

HBV uses reverse transcription to copy its DNA genome and lack of proof-reading capability permits the emergence of mutations in the genome. Every day, approximately 10^{11} viral particles are produced and released to maintain a stable level of virus in blood. The average mismatch rate of HBV polymerase is from $1:10^5$ to $1:10^4$, potentially resulting in a large amount of mutants in the circulation [13–16].

For single individuals infected with HBV, HBV genome mutation either naturally occurs naturally or is selected out by antiviral drugs or the change of internal host environment. The probability of HBV mutations varies in different regions of the whole genome. Generally, mutations are more likely to occur in the basal core promoter (BCP), pre-C region and neutralization determinants of the viral envelope, but they can also be found in other regions. Some of these mutations have important clinical significance, but most of them are silent mutation without biological significance.

1.5.2.1 Pre-C/BCP Gene Mutation

Pre-C region encoding HBeAg, is composed of 29 amino acids. The most common mutation is a guanine (G) to adenine (A) substitution at nucleotide 1896, which creates a premature stop codon at codon 28, and prevents the translation of the HBeAg [17]. Although synthesis of HBeAg is inhibited, the HBV replication still continues, manifesting as HBeAg negative HBV infection. HBeAg expression is not necessary for viral replication, its role in the HBV life cycle remains unclear. In the immune system, HBeAg may act as bait, which induces immune tolerance, especially in the newborn babies whose mothers have high level viremia. The HBeAg-induced immune tolerance can prevent the attacks on the virus-infected hepatocytes by CTL on HBcAg, thus the HBV-infected hepatocytes are not able to be cleared. The hindered synthesis of HBeAg may facilitate the CTL to damage infected liver cells, which might be one of the mechanisms of severe hepatitis. It is reported in Japan that patients, especially children, infected with this mutant, are more likely to suffer from severe hepatitis. Other pre-C mutations include point mutations generating other termination codons, for example G1897A generating UGA codons. Point mutations may change the initiation codon of P25 or the specific amino acid for cleavage and insertion of key signal peptide, particularly between 1838 and 1839. All of these mutations can affect the HBeAg production. The pre-C mutation can reduce HBeAg expression, increase HBV replication and aggravate liver damage [18]. A number of studies have suggested the association of pre-C mutation and development of severe hepatitis. The HBcAg encoded by C region, contains a

T-cell-dependent/independent epitopes and induces the host humoral and cellular immune responses. The C gene mutation may cause deletion of the cell surface antigen, and consequently the lack of humoral immune response by the host against HBV. However, the cytotoxicity of CTLs was not affected resulting in potentially massive necrosis of liver cells and eventually leads to severe hepatitis.

Core promoter directly activates pgRNA transcription, and plays a central role in HBV replication. Core promoter is composed of basic core promoter (BCP) and its upstream regulatory elements. The BCP region partly overlaps with the 3 'end of X gene and the 5' end of pre-C gene and can independently start the transcription of pre-mRNA and pgRNA. The core promoter mutation, which is associated with Nt1758–1762 that are at the upstream of the starting point of pre-C mRNA, reduces the synthesis of HBeAg, but has no effect on HBcAg. The development of severe hepatitis is often accompanied with HBV core promoter mutation, especially the 1762,1764 double set of mutations [19]. Other common mutations in the core promoter region of patients with severe hepatitis include nt1768 C-T, nt1770 T-A and cluster mutations, including nucleotide insertion, deletion and substitution.

The BCP mutations can induce changes of two codons (L130M and V131I) in X protein, and generate the HNF1 (hepatocyte nuclear factor 1) binding sites. Insertion of 11 bases into the core promoter produces a new binding site of HNF1, which can enhance viral replication and lead to severe hepatitis [20]. In-vitro studies have confirmed that BCP trans-activating transcription causes X protein replacement, which downregulates the transcription of pre-C region and pgRNA [21]. However, the double-mutant can upregulate the transcription of pgRNA, increase HBcAb production, thereby enhancing viral replication. Previous studies have shown that A1762T and G1764A mutation are more prevalent in HBV genotype C, which partially explains the stronger pathogenicity of genotype C than genotype B. Recent studies have shown that A1762T and G1764A mutations in HBV genotype B may be associated with severe hepatitis, but in HBV-infected pediatric patients, A1762T and G1764A mutations show no significant difference in genotype B and C. Overall though, compared with wildtype HBV, BCP double mutation is more commonly associated with severe liver diseases, especially liver cirrhosis and HCC [22].

The mutations in pre C/BCP region may alter the biological characteristics of the virus, and induce the development of severe hepatitis through impacts on host immune responses, being more vigorous in HBeAg-negative CHB [23]. However, the biological significance of mutations in different sites or forms, the dynamic interaction between virus mutation and host immunity, the influence of different genotypes and viral quasispecies on mutation are still not fully elucidated.

1.5.2.2 Pre-S/S Gene Mutation

Immune evasion or vaccine failure related pre-S point mutation or deletion mutation does not affect the viral replication. The pre-S1, S2 recombination, including deletion mutation and promoter mutation, have been regularly found in patients either with chronic or fulminant hepatitis. These mutations frequently occur after HBeAg seroconversion or interferon treatment, suggesting the host immune selective pressure during their selection. The Pre-S region mutations may play a role in HBV persistent infection, and may also cause liver damage. It has been reported that the

pre-S2 mutation is related to fulminant hepatitis, and the pre-S mutation is a strong risk factor for the development of HBV-related liver cancer [24, 25], presumably as a result of an accumulation of viral envelope proteins inside the cell.Mutations of the surface antigen protein, particularly AA145 mutation (sG145R), result in conformational change of the major antigen epitope 'a'. This change disables the immunological recognition and surveillance of the host immune system for the mutant can also result in failure of the clinical vaccination and might be one of the precipitations for the exacerbation of hepatitis B [26].

1.5.2.3 Nucleos(t)ide Analogues-Induced P Gene Mutation

The P gene mutation in key catalytic domains indicates the HBV resistance against nucleos(t)ide analogues (NA). The current five NAs in clinical application include lamivudine, adefovir, telbivudine, entecavir and tenofovir. The clinically extensive application of NAs leads to rapid selective drug resistance. The selection of drug-resistant mutation depends on the following factors: (1) the long half-life of hepatocytes and intrahepatic cccDNA; (2) the capacity of HBV replication and mutation; (3) antiviral drug pressure; (4) genetic barrier to resistance. For example, the lamivudine resistance is closely associated with the HBV reverse transcriptase gene YMDD motif mutation, presenting as rt M204I and rt M204V mutation, with or without RT L180M mutation. The replication capacity of rt M204I/V mutants is weaker than the wild strains in the absence of drug. The rtL180M mutation can restore the HBV replication capability. In addition, the single or combined mutation of rt L80I, rt L82M, rt F166L, rt V173L, rt A200V and rt V207I may compensatorily restore the replication capability of rtM204 I/V mutant [27]. The resistance rate is up to 40% after 2-years of Lamivudine therapy, and more than 76% after 5-year mono-therapy. The 1-year resistance rate of telbivudine is about 7%. The rtN236T site mutation (threonine substitute asparagine) is mainly seen in adefovir resistance, and the 5-year resistance rate is about 29%. The rtA181T/V (valine/threonine substitution alanine) site mutation is found in all the above three antiviral drugs. Entecavir resistance mutations include 204 and 180 substitutions, combined with substitutions at codons 184, 202 and 250. The 4-year resistant rate in patients initially treated with entecavir is less than 1%. For patients that have been previously treated with lamivudine, the resistance rate of entecavir increased significantly. So far, there have been no reports on the primary drug resistance for tenofovir mono-therapy. The rtA194T mutation was found in the combination therapy of tenofovir and lamivudine [28].

The entire S gene is included in the P gene, and the RT region overlaps with S gene, thus the RT mutation may cause the S gene mutation. Double mutations in these two regions can change the viral replication capacity. The HBV drug-resistance mutation occurs in patients with chronic hepatitis B may reduce the HBeAg seroconversion rate, reverse the histological improvement, increase the disease progression rate, aggravate liver cirrhosis, and increase the death risk of liver transplant patients. RT mutations may also cause the S protein epitope changes and affect the HBs antibody and the function of CTL, suggesting its role in the development of liver failure [29, 30].

As salvage therapy for lamivudine resistance, lamivudine combined with adefovir dipivoxil has higher rate of viral suppression and lower rate of adefovir

resistance compared with switching to adefovir dipivoxil monotherapy [31, 32]. Tenofovir is a potent antiviral drug for lamivudine-resistance salvage therapy, and showed a better effect than switching to adefovir dipivoxil monotherapy [33]. In contrast, switching to entecavir is not an optimal choice for lamivudine-resistance [34]. Telbivudine resistance is associated with the rtM204I mutation, and has cross-resistance with lamivudine. Therefore, telbivudine could not be an alternative for lamivudine-resistance [35]. Treatment for telbivudine resistance is similar to that for lamivudine resistance [36].

Treatment for adefovir-resistance is determined by the virus mutation types and antiviral medication history [37, 38]. Lamivudine has been proved to be effective on inhibiting rtN236T adefovir resistance mutations [39]. In vitro data has also suggested the effectiveness of telbivudine. Additionally, entecavir might be a reasonable choice for rtN236T mutants. Patients with rtN236T mutation are suggested to (1) switch to or add entecavir; or (2) add lamivudine or telbivudine; or (3) switch to tenofovir.

Compared with that on HBV wild strain, lamivudine becomes less effective on A181V adefovir resistant strain. In vitro studies show that tenofovir has reduced sensitivity to the rtA181T mutation. Clinically, entecavir and tenofovir can effectively inhibit the replication of A181T adefovir resistant mutants [40]. Patients with rtA181T mutation are suggested to (1) switch to or add entecavir; or (2) switch to tenofovir. Under this circumstance, lamivudine should not be suggested in case it increases the risk of cross-resistance [41, 42].

Currently, there has been no data from large sample clinical trials that can guide the treatment for entecavir resistance. In-vitro studies and case reports suggested that adefovir and tenofovir are effective for entecavir-resistant patients. Based on expert opinions, patients with entecavir resistance are recommended to add tenofovir or adefovir [43–45].

In summary, the virus mutation in above regions may be associated with the pathogenesis of severe hepatitis. However, the severity of hepatitis depends on key factors of virus and host. The same mutant may lead to different clinical outcomes in different hosts. Thus except for virus mutation, factors including host immune status, cytokines and HLA might also account for the development of severe hepatitis.

1.5.3 Serological and Virological Characteristics and Clinical Tests for HBV Infection

1.5.3.1 Detection for HBV Serum Immunological Markers

HBV Surface Antigen/Pre-S1 Protein/Pre-S2 Protein

HBsAg is the major coat protein of HBV with antigenicity but not infectivity. In a broad sense, HBsAg contains the major protein, middle protein and large protein. Narrowly, HBsAg simply refers to the major protein, which appears earliest and has the highest titer. Thus it is considered as an important marker for early diagnosis of hepatitis B. For typical acute hepatitis B, HBsAg appears during the incubation period, followed by the clinical symptoms and abnormal liver function

in 2–6 weeks. HBsAg stays in the blood for 1–2 months, and disappears in the recovery period. Persistence of HBsAg more than 6 months indicates the development of chronic hepatitis. HBsAg can also be detected in HBV carriers and patients with HBV-related liver cirrhosis or liver cancer. A rapid reduction of quantitative HBsAg within 3 months can predict the efficacy of antiviral drugs, but no changes in the quantitative HBsAg level after 6 months is not considered as a good predictor [46, 47]. Pre-S1 protein, which appears early and is significantly associated with HBeAg and HBV DNA, can be used as a marker of acute hepatitis B early in infection. The Pre-S1 protein has a strong immunogenicity, and includes the important site where HBV attaches to and invade the hepatocytes, the sodium taurocholate co-transporting peptide (NTCP). It is also a reliable reflection of HBV replication level.

The synergy from pre-S2 protein is also important for HBV invasion. Most patients with acute exacerbation of chronic hepatitis, or chronic active hepatitis, or acute hepatitis developing to chronic hepatitis have persistent pre-S2 protein expression in serum. Therefore, serum pre-S2 protein can be used to estimate the activity and infectivity of HBV in chronic patients [48]. Pre-S1 and pre-S2 protein, which can induce and regulate humoral and cellular immune response of the host, provide important immune defense for eliminating virus in blood circulation and preventing healthy liver cells being infected.

HBV Surface Antibody (HBsAb)

HBsAb, which is a protective antibody, can eliminate the virus and prevent HBV infection. HBsAb appears in the late stage of acute infection, just before HBsAg becomes negative, and will gradually rise to the peak levels in 6–12 months. This antibody can last for a long time, but the titers will gradually decline after 10 years. A small number of cases do not produce HBsAb after HBsAg becomes negative. In acute hepatitis B infection, appearance of HBsAb suggests recovery of the disease. Patients with severe hepatitis often present with high titers of HBsAb, which forms the immune complexes with HBsAg, which can induce flares of hepatitis that lead to liver cell necrosis. After the hepatitis B vaccination, HBsAb (>10 IU/L) means the successful vaccination and development of immunity.

Hepatitis B Core Antigen (HBcAg)

In the blood, HBcAg is mainly located in the core of the Dane particles or virions. The only small amount of free HBcAg is also combined by high titers of HBsAb and presents as immune complexes. Thus, it cannot be detected unless treated by detergent. HBcAg on the surface of hepatocytes is considered to be the main target antigen of the host CTLs. HBcAg is a direct evidence of HBV infection and replication, and also a marker for evaluating the efficacy of antiviral drugs.

Hepatitis B Core Antibody (HBcAb)

HBcAg is strongly immunogenic, so that HBcAb can be detected in most patients with HBV infection. HBcAb often emerges in the early stage after infection, is present in high titer in blood and can persist for a very long time. Titer of HBcAb above 1: 100,

together with abnormal ALT level, can be used for the diagnosis of hepatitis B infection. For occult hepatitis B, high titer of HBcAb is also valuable for the diagnosis. HBcAb consists of IgM and IgG antibodies. Anti-HBc-IgM, which suggests HBV-resulted liver damage, is the main evidence for the diagnosis of acute hepatitis B. It may become positive during the active phase of chronic hepatitis B and turn negative during remission. It also appears during the flares of chronic hepatitis B, mostly in a week after infection, and disappears within 6 months. Anti-HBc-IgG appears late but is sustained for many years or even a lifetime. In patients with acute hepatitis B, the titer of anti-HBc-IgM is higher than anti-HBc-IgG, while in those with chronic hepatitis B the situation is opposite. Both antibodies show high titers in fulminant hepatitis.

Hepatitis Be-Antigen (HBeAg)

HBeAg is a soluble antigen, which appears later than HBsAg. Sustained expression of HBeAg suggests persistence of HBV infection. In patients with chronic Hepatitis B, HBeAg acts as an important immune tolerance factor leading to a low immune response to HBV infection. It is a valuable marker for evaluating the efficacy of antiviral drugs.

Hepatitis Be-Antibody (HBeAb)

Seroconversion refers to the loss of HBeAg and development of anti-HBe. Approximate 2–15% patients have spontaneous seroconversion every year. Studies have shown that spontaneous seroconversion occurs earlier in patients with genotype A, B, D and F than those with genotype C.

Appearance of HBeAb demonstrates the decrease or termination of viral replication and low infectivity. Recent studies showed that, after 1 year since the spontaneous HBeAg seroconversion, viral load more than 2000 IU/mL increased the incidence of fulminant hepatitis.

For HBV chronic carriers and patients with HCC, HBeAb does not mean the recovery, or elimination. In contrast, HBV DNA integration is often found in these patients.

X Protein/Anti-HBx

X protein is capable of transactivating the expression of numerous cellular and viral genes, and is vital for virus replication. X protein can be detected in some patients with chronic hepatitis, so it is used as an auxiliary diagnostic marker of HBV infection [49]. X protein plays a central role in HBV-related HCC progression and stimulation. Thus, follow-up is necessary for patients active and persistent HBV replication.

1.5.3.2 HBV DNA Quantitative Detection

Serum HBV DNA is the direct evidence of active HBV infection, reflecting the level of viral replication and infectivity. The quantitative detection for viral genes is a very important marker for treatment decision, efficacy prediction and observation. Long-term high load of HBV DNA is an independent risk factor for predicting the development of liver cirrhosis and HCC. Numerous studies have shown that viral load is the most reliable marker to predict the development of HCC [50].

1.5.4 Detection for HBV Quasispecies

1.5.4.1 Research Progress on HBV Quasispecies

The differences of HBV whole genome sequence are approximately 2–4%. The diverse variants that are genetically linked through mutation are known as quasispecies. Quasispecies contain a large number of mutated genes serving as a reservoir for viral selection under the pressure of immune response and antiviral treatment. When changes occur in the environmental conditions, the quasispecies structure responds by rebalancing its composition. The predominant sequence may shift by selection of a variant that is better adapted to the new environment, in the classic Darwinian process of survival of the fittest [51]. This feature gives the virus strong adaptability, and makes it difficult to prevent and control. Study on the relationship between quasispecies and different clinical outcomes will provide valuable information for exploring anti-HBV treatment strategies.

Quasispecies Evolution Under Different Environment

1. Quasispecies evolution under host immune pressure.

In chronic HBV infection transmitted via perinatal transmission, the different immune phases of chronic HBV infection confer different environments on HBV quasispecies. Thus, the characteristics of HBV quasispecies may differ. A preliminary study on the differences of full-length HBV quasispecies between mothers and their progeny showed that, after 30 years of evolution, the dominant sequence of HBV quasispecies became different between mothers and daughters. The characteristics of HBV quasispecies in various gene regions are different in mothers and daughters with different treatment responses or disease status. Among these genes, the preC/C gene had the highest substitution rate [52].

2. Quasispecies mutation under pressure of antiviral drugs.

Under antiviral drug selection pressure, HBV mutants are selected from the preexisting pool of quasispecies and over time become the dominant species [53]. The probability of resistant mutations depends on the effectiveness of antiviral drugs. Low potent drugs have almost no selective pressure on the virus, thus lead to low probability of viral resistance; on the contrary, drugs that completely inhibit viral replication also rarely result in resistance. Only medium potent drugs have the highest rates of drug resistance.

A study showed that during the 4-week lamivudine therapy, distinct patterns of quasispecies evolution are found between responders and non-responders; the structures of viral quasispecies tended to be simpler in responders, but more complicated (higher diversity) in non-responders. Similar phenomenon was also observed during entecavir therapy. Another study detected the full length sequence of resistant virus in lamivudine and adefovir sequential therapy, and found that variation of nucleotide or amino acid sequence usually occurs in HBV HBsAg or RT region.

Quasispecies and Clinical Outcome

Using single strand conformation polymorphism (SSCP) and DNA sequence analysis, researchers found that the complexity of HBV quasispecies in patients with cirrhosis was more than those in patients with chronic hepatitis B, suggesting that complexity of HBV quasispecies is associated with disease status [54]. Researchers from China had also used the same methods to analyze the difference of quasispecies complexity in S region among patients with chronic severe hepatitis B, patients with chronic hepatitis B and HBV carriers. It is found that the quasispecies complexity in the S region increases along with disease progression in chronic HBV infection. Analysis on quasispecies in acute hepatitis B, chronic HBV carriers, chronic hepatitis B and chronic severe hepatitis by full-length HBV genomic clone and bioinformatics methods also discovered the positive correlation between HBV quasispecies complexity and disease severity [55]. On one hand, complex evolution of quasispecies may lead to persistent infection and continuous liver damage, and increase opportunities for the emergence of new HBV variants. On the other hand, enhanced virulence of mutated virus and change of antigen epitope may cause excessive immune response and severe liver damage. However, correlation between HBV quasispecies complexity and disease severity still needs dynamic large sample research to confirm [56].

HBV Quasispecies and Antiviral Efficacy of Nucleos (T) Ide Analogs

The dynamic change of quasispecies during NA antiviral therapy may be related to the antiviral efficacy and drug resistance. Results from a study on patients receiving lamivudine for 48 weeks suggested that the baseline quasispecies heterogeneity is not associated with antiviral efficacy, and the changes of quasispecies complexity at an early stage may predict antiviral efficacy and drug resistance more accurately than the change of HBV DNA level during lamivudine therapy. Another study on dynamic changes of quasispecies in patients receiving entecavir antiviral therapy suggested that, compared to the partial responders, quasispecies complexity is reduced but dispersion is increased in complete responders after 4 weeks of treatment [57]. In these two studies, the quasispecies dispersion decreased in lamivudine responders but increased in entecavir responders after 4 weeks of treatment. This might be because entecavir has stronger antiviral effect than lamivudine, thus generates more selection pressure. In addition, entecavir can still induce complete response even when some mutations occur since it has a higher drug resistance barrier. Therefore, the early changes in HBV quasispecies complexity may act as a predictor of sustained antiviral effect of nucleos(t)ide analogues.

The resistant strains typically already exist before antiviral therapy, and become the predominant strains under selection pressure of antiviral drugs. A study showed that adefovir treatment for 240 weeks in patients with chronic hepatitis B selected resistant virus strains [58]. Study on gene heterogeneity of HBV reverse transcriptase suggested that lamivudine monotherapy is liable to induce the quasispecies that affect response rate of salvage therapy with adefovir for virologic breakthrough in lamivudine-treated patients, and reduce the sensitivity to other nucleos(t)ide analogues [59].

Under different drug selection pressure, non-responders have similar quasispecies evolution patterns, suggesting that this pattern may be associated with viral resistance mechanisms. From the perspective of quasispecies, drug selection pressure changes the relative ratio of viral populations, and leads to population drift.

1.5.4.2 Detection Technology for Quasispecies Mutation

DNA Sequence Analysis

DNA sequence analysis is the most direct and reliable method to detect gene mutations. It is also the gold standard to detect nucleic acid sequence mutation. However, the high cost of this method prevents its application in large sample research. DNA sequencing, which can be divided into direct sequencing and cloning sequencing, is built on the basis of high-resolution denaturing polyacrylamide gel electrophoresis. Direct sequencing detects the nucleotide sequence of dominant strains; clone sequencing method can find out changes of nucleotide in other strains.

Restriction Fragment Length Polymorphism (RFLP)

RFLP is a technique that applies to detect variations in homologous DNA sequences. It refers to a difference between samples of homologous DNA molecules from differing locations of restriction enzyme sites, which may be naturally formed or brought in by PCR mismatch. The DNA sample is broken into pieces and digested by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. Mismatched PCR is a modified PCR, which changes one or a few nucleotide bases in designed PCR primers to make the synthetic DNA meet special requirements. Here, a restriction enzyme recognition sequence is introduced into the PCR amplified fragment in order to change the target DNA restriction map and distinguish the mutants and non-mutants.

Nucleic Acid Hybridization

Nucleic acid hybridisation is the pairing of complementary single-stranded nucleic acids (DNA or RNA) to produce DNA–DNA or DNA–RNA hybrids. When the target DNA obtained by PCR amplification combines with the probe labeled by radioactive or non-radioactive labels, any mismatch between the probe and target DNA can be detected. This helps to distinguish the wild and mutant strains. Nucleic acid hybridization applies to high frequency point mutation and is suitable for large sample detection, but the high requirement for hybridization temperature makes it difficult to popularize.

Single-Strand Conformation Polymorphism (SSCP)

The principle of SSCP is based on conformational difference of single-stranded nucleotide sequences of identical length. This property allows sequences to be distinguished by means of gel electrophoresis so as to determine whether mutations exist. It applies to the single base substitution and screening of DNA fragment mutation. However, SSCP is neither stable nor practical [60, 61].

3′ Base-Specific PCR

Primer extension starts from the 3′ end in PCR amplification, and only succeeds when the 3′ end of the primer is an exact match to the template DNA. Based on this phenomenon, the primer with 3′ end containing mutated bases is used to detect the mutation of target DNA. The 3′ base-specific PCR technology is simple but with low sensitivity, and the results are prone to be false-negative. The incomplete block of PCR amplification also leads to false-positive results [62].

Melting Curve Analysis

Melting curve analysis is an evaluation of the dissociation-characteristics of double-stranded DNA during heating, the temperature at which 50% of DNA is denatured is referred to the melting point [63]. In chronic HBV infection, the peak numbers of DNA melting curve in patients with moderate and severe hepatitis are significantly more than those in HBV carriers and mild hepatitis; the peak number of melting curve in patients with severe hepatitis is significantly more than that in moderate hepatitis. The melt-curve analysis is less sensitive than SSCP, but is more accurate on analyzing genetic variation. The strong operability and high cost-effective make it a preferable method for genetic variation analysis.

The Next-Generation Sequencing Methods

The high demand for low-cost sequencing has caused the development of high-throughput sequencing—the next-generation sequencing, which includes massively parallel signature sequencing (MPSS), Solexa sequencing, SOLiD sequencing, 454 pyrosequencing, HeliScope single molecule sequencing, etc. These methods apply to genome sequencing, genome resequencing, RNA-Sequencing, ChIP-sequencing and epigenome characterization [64–66].

Third-Generation Sequencing Methods

Current DNA sequencing methods under development include microscopy-based techniques, macromolecule and nanotechnology that can distinguish the base signal and directly read the sequence without the use of biological or chemical reagent. The third-generation sequencing methods include: (1) non-optical microscope imaging: the DNA sequence can be read out if the resolution of image is high enough to differentiate the four kinds of bases on DNA when visualizing the spatial linear arrangement of nucleotides. This idea is based on non-optical microscope at the atomic level, for instance, scanning tunneling microscopy (scanning tunneling microscope, STM) [67]. (2) Nanopore DNA sequencing: It is based on the readout of electrical signals occurring at the single stranded DNA or RNA molecules passing by alpha-hemolysin pores covalently bound with cyclodextrin [68–70]. Those methods still need further validation and improvement.

1.5.5 Summary and Outlook

The pathogenesis of HBV infection is determined by the interplay between both virus and host. Different outcomes after infection are related to different host immune responses and viral mutations. However, the biological significance of viral

mutation has not been fully elucidated because of: (1) the limited samples and lack of comparison between different groups; (2) the overlap of each HBV genomic coding regions; (3) the limitation of detection technology. With the rapid development of detection technology, the large sample, long-term, multi-level studies will help to understand more about the host-virus interaction and potential mechanisms.

1.6 Clinical and Laboratory Parameters for Liver Transplantation for Hepatitis B-Induced Liver Failure

Zhi-Liang Gao, Liang Peng

Liver transplantation is now the most effective therapy for the treatment of irreversible acute and chronic end stage liver disease. Since Dr. Thomas Starzl performed the first orthotopic liver transplantation in humans in 1963, liver transplantation has now developed rapidly largely as a result of advances in surgery, transplant immunology, anesthesiology, and the development of specialized units to provide care for these desperately ill patients. In Mainland China, liver transplantations began in the 1970s, with the first liver transplant performed in Shanghai Ruijin Hospital in 1977. Over the past 3 decades, China has been home to the second largest number of patients receiving liver transplantation worldwide. According to statistics from the China Liver Transplantation Registry (CLTR) in 2006, the 1-year and 5-year survival rate of patients receiving liver transplantation is 80.5% and 65.9%, respectively. These data demonstrate that clinical efficacy of liver transplantation in China is similar to international standards.

To standardize and improve the clinical application and management of organ transplantation in humans and to ensure that the interests and medical safety of patients are met, Professor J-F Huang, a deputy minister of the Ministry of Health, sponsored the drafting of Interim Provisions for the Clinical Application and Management of Organ Transplantation in Humans in July 2006, which was issued. In November 2006, the Summit of Clinical Application and Management of Organ Transplantation in Humans was held in Guangzhou, China, in which delegates mandated the national medical profession to abide by the medical guidelines, ethical principles, and professional ethics for respecting life and resolutely resisting all forms of human organ trafficking activities. On March 21, 2007 the State Council issued the Regulations for Human Organ Transplantation, which was implemented on May 1, 2007. Those measures were praised by the World Health Organization and The Transplantation Society, symbolizing a key step in the legalized and standardized development of organ transplantation in China.

1.6.1 General Indications for Liver Transplantation

The indications for liver transplantation are changing over time. Before 1980, the major indication for liver transplantation was primary malignancy of the liver, especially hepatocellular carcinoma (HCC) which significantly influenced the

Table 1.7 Major indications for liver transplantation

Classification	Specific causes
End-stage liver disease	Chronic severe viral hepatitis (B or C) Posthepatic cirrhosis (decompensated) Primary sclerosing cholangitis Primary biliary cirrhosis Alcoholic cirrhosis Autoimmune hepatitis
Liver tumors	Malignancies Multiple hepatic adenomas Large hepatic hemangioma Intrahepatic metastatic neuroendocrine tumors
Acute liver failure	Viral hepatitis (B or C) Toxicity (drugs, toxin, or food)
Congenital or metabolic diseases	Congenital biliary atresia Wilson's disease Cystic dilatation of the intrahepatic bile ducts Glycogen storage disease Hemochromatosis α1-trypsin deficiency Polycystic liver
Other	Cryptogenic cirrhosis Budd-Chiari syndrome Trauma Intrahepatic bile duct stones

Redraw from Cai CJ, Lu MQ, editor. Peri-operative therapies of patients receiving liver transplantation due to severe hepatitis. Sun Yat-sen University Press, Guangzhou, China; 2008. ISBN: 9787306031860 [71]

therapeutic efficacy of liver transplantation and postoperative survival. Currently, not only are acute or chronic liver diseases nonresponsive to other medical treatment and surgery now the major indication for liver transplantation but also liver diseases that markedly affect quality of life. The major indications for liver transplantation are shown in Table 1.7.

1.6.2 Current Status of Liver Transplantation Due to Hepatitis B

Viral hepatitis has and continues to be a major public health problem in China. It is the most common liver disease and most common cause of liver failure. The proportion of patients with hepatitis B-induced liver failure among patients receiving liver transplantation in China is significantly higher than in other countries.

Hepatitis B has diverse clinical manifestations and may progress to chronic hepatitis, liver failure (including acute liver failure, acute on chronic liver failure, and chronic liver failure), hepatic cirrhosis (compensated and decompensated), and even hepatitis B-related liver tumors. Whether liver transplantation is necessary for patients with HBV infection and the timing of transplantation must be carefully considered. Early liver transplantation may increase patient risk and be an economic

burden because patients could recover and even survive for a long time in the absence of liver transplantation. However, presently it is difficult to predict patient outcome to HBV infection. In contrast, if liver transplantation is performed in the late stages of disease, numerous complications of hepatitis B-induced liver dysfunction may either increase the risk of transplant surgery resulting in a poor prognosis. Thus, for patients with severe hepatitis B, the indications for and the timing of liver transplantation are crucial and should be carefully evaluated.

1.6.3 Clinical and Laboratory Indications for Liver Transplantation in Hepatitis B Patients

In general, the following conditions are indications for liver transplantation in patients with chronic hepatitis B:

1. Obvious manifestations of liver failure including sustained elevation of serum bilirubin to >5 mg/dL; prothrombin time > 5 s longer than the reference range; plasma albumin <2.5 g/dL; and liver failure which is nonresponsive to active and/or symptomatic therapy (such as infusion of fresh plasma and albumin) or if the patient continues to deteriorate clinically despite optimal medical therapy.
2. When there are complications related to either liver failure or portal hypertension such as the presence of severe hepatic encephalopathy, disturbances of coagulation function, refractory bleeding due to rupture of esophageal or gastric varices, refractory ascites, repeated episodes of spontaneous bacterial peritonitis, or the development of hepatorenal syndrome.
3. When hepatitis B influences the quality of life including the development of severe lethargy, uncontrollable itching, metabolic bone diseases or the development of bacterial cholangitis and
4. The development of hepatocellular carcinoma.

Although there are some well defined indications for liver transplantation, the ultimate determination of the timing and specific indications for liver transplantation remains a challenge in clinical practice for hepatitis B patients with liver failure. The introduction of the Model of End Stage Liver Diseases (MELD) and some derivative scoring systems are now frequently used to determine whether a patient should be listed for transplantation and the urgency of liver transplantation.

In 1999, the Mayo Clinic proposed that MELD should replace the Child-Pugh grading system for the determination of urgency of liver transplantation. MELD is calculated using the following calculation:

MELD = $9.6 \times \log_e$ (creatinine [mg/dL]) + $3.8 \times \log_e$ (bilirubin [mg/dL]) + $11.2 \times \log$ (INR) + $6.9 \times$ (causes: 0 for biliary and alcoholic; 1 for others).

MELD was originally used for the evaluation of prognosis of hepatic cirrhosis patients receiving transjugular intrahepatic portosystemic shunt. As described above, the MELD score is determined on the basis of total bilirubin, international standardization of bleeding time, serum creatinine, and etiology. Compared to the

Child-Pugh grading system, MELD employs objective parameters, which are helpful for the comparisons of patients from different centers. While the MELD score changes with the alteration of the liver disease, the Child-Pugh grade has only three levels and is unable to meet the requirements of accurate and objective evaluation. Saab et al. investigated the prognosis of 404 patients receiving liver transplantation and found that the 1-year survival rate was 90, 89, 90, 79, and 69% in patients with preoperative MELD scores of ≤10, 11–18, 19–24, 25–35, and ≥36, respectively. However a higher MELD score negatively affected the 1-year survival rate. For example patients with a MELD score ≤24 had a 1 year survival rate of 88 whereas patient survival was reduced to 65% in patients with a MELD score >24.

Despite the benefits and the widespread application of MELD in clinical practice, there remain some imperfections in the evaluation of timing of liver transplantation and the assessment of prognosis and therapeutic efficacy. One of the major imperfections is the failure to consider complications such as infection, hepatic encephalopathy, hepatorenal syndrome, and disturbances in fluid and electrolytes which may significantly influence the prognosis and timing of liver transplantation but are not included in the MELD scoring system. Hence inclusion of sodium (Na) in the MELD scoring system:

MELD − Na [MELD + 1.59 × (135 − Na)], iMELD [MELD + (0.3 × age) − (0.7 + Na) + 100]; and MESO [MELD/Na (mmol/L)] × 10 has improved outcomes of patients requiring liver transplantation.

Those scoring systems, however, also fail to consider other complications. Including the development of hepatic encephalopathy or ascites and therefore, their applicability is limited in China.

In China, liver transplantation was initiated relatively late compared with other countries; regulations regarding liver transplantation remain imperfect; and smooth communication among transplantation centers in different regions of the country are lacking. These drawbacks markedly influence the timely and fair distribution of donor livers. Determination of waiting times for transplantation is not scientific. Therefore, the comprehensive evaluation of a patient's condition of hepatitis B-induced liver failure, determination of the timing of liver transplantation, and optimization of the rational and fair distribution of donor livers needs to be carefully considered by Chinese clinicians in the field of hepatology going forward if improvements are to occur.

There are currently additional scoring systems which have been used by Chinese clinicians. One of these was developed by Professor Ke WM in the Department of Infectious Diseases of the Affiliated Third Hospital of Sun Yat-sen University. In that system, hepatic encephalopathy, serum creatinine, prothrombin activity, serum total bilirubin, liver size (determined by ultrasonography), amount of ascitic and pleural fluid (determined by ultrasonography), and infection (peripheral white blood cell count, proportion of neutrophils, and inflammatory findings from thoracic imaging examinations) are taken into account and objectively and conveniently scored on a scale of 0–4 according to their severities as described in Tables 1.8, 1.9, and 1.10.

Table 1.8 Scoring system for the evaluation of prognosis of acute on chronic liver failure in hepatitis B patients

Score (point)	HE stage	Tbil	Maximum ascitesfluid level (mm)	PTA (%)	Right hepatic lobe		Serum Cr	Infection
					Oblique diameter	Vertical diameter		
1	I	10–20 ULN	0–40	30–40	110–120	100–110	1.0–1.1 ULN	WBC 10×10^9/L-15×10^9/L or N 0.7–0.8
2	II	20–30 ULN	40–80	20–30	100–110	90–100	1.1–1.2 ULN	WBC 15×10^9/L-20×10^9/L or N 0.8–0.9
3	III	30–40 ULN	>80	10–20	90–100	80–90	1.2–1.3 ULN	WBC $>20\times 10^9$/L-or N > 0.9
4	IV	>40 ULN	Ascites with unilateral or bilateral pleural effusion	<10	<90	<80	>1.3 ULN	Image changes indicating Lung inflammation

ULN upper limit of normal. A score 1–3 is determined on the basis of either peripheral white blood cell count or the proportion of neutrophils reaching the threshold firstly. A score of 4 is determined according to the inflammatory findings of thoracic imaging (not including peripheral white blood cell count and proportion of neutrophils)

Redraw from Liu TH, Zhu JY, Zhang SQ, et al. Establishment of a scoring system for evaluating the severity of hepatitis B patients with acute-on-chronic liver failure. Chinese J Infect Dis 2010;28(5):293–6. (Article in China) [72]

Table 1.9 Severe clinical parameters used for the evaluation of acute on chronic liver failure in living or deceased hepatitis B patients

Group	Case number	PTA score	Serum Cr score	Infection score	HE score	Tbil score	Liver size score	Ascites score
living group	203	1.63 ± 0.78	0.20 ± 0.78	0.82 ± 1.31	0.58 ± 2.89	1.36 ± 0.81	2.26 ± 0.86	1.34 ± 1.17
deceased group	196	2.62 ± 0.80	2.04 ± 1.91	2.20 ± 1.45	2.85 ± 1.42	2.10 ± 0.92	2.86 ± 0.95	2.12 ± 1.23
t value	–	12.600	12.561	9.950	9.878	8.557	6.659	6.475
p value	–	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Redraw from Liu TH, Zhu JY, Zhang SQ, et al. Establishment of a scoring system for evaluating the severity of hepatitis B patients with acute-on-chronic liver failure. Chinese J Infect Dis 2010;28(5):293–6. (Article in China) [72]

Table 1.10 Scores of acute on chronic liver failure determined according to a new scoring system and MELD in either living or deceased hepatitis B patients

Group	Case number	New scoring system	MELD score
Living group	203	8.07 ± 3.14	26.43 ± 5.58
Deceased group	196	16.91 ± 3.54	40.16 ± 10.22
t value	–	26.125	16.566
p value	–	<0.01	<0.01

Redraw from Liu TH, Zhu JY, Zhang SQ, et al. Establishment of a scoring system for evaluating the severity of hepatitis B patients with acute-on-chronic liver failure. Chinese J Infect Dis 2010;28(5):293–6. (Article in China) [72]

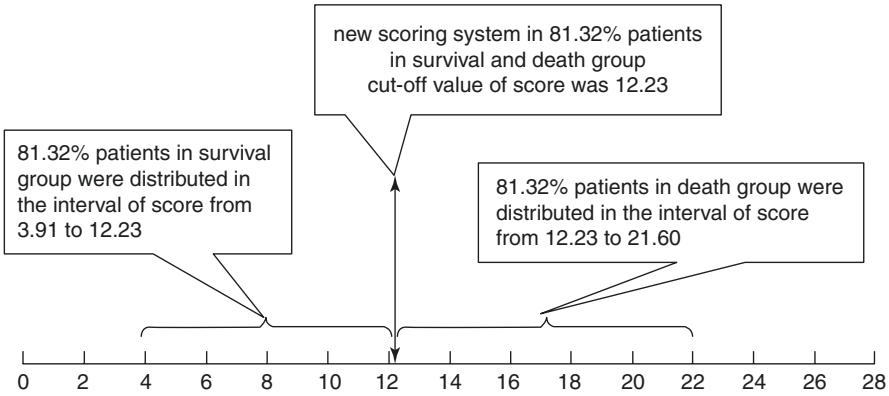

Fig. 1.7 Cut-off value and distribution range of total score determined with a new scoring system in 81.32% of surviving and deceased patients

These newer scoring systems and the MELD scoring system can favorably predict the mortality of acute on chronic liver failure patients with hepatitis B. The area under receiver operator curves of scores determined with a new scoring system and MELD scoring system was 0.960 [95% confidence interval (CI), 0.944–0.977] and 0.886 (95% CI, 0.852–0.920), respectively. There was no overlap in 95% CIs between the two, and they were significantly different ($P < 0.01$) as illustrated in Figs. 1.7, 1.8, and 1.9).

Wenzhou Medical College in China subsequently proposed a scoring system in which the total score is calculated using the following equation:

$X = 1.4053 + 3.6017 \times$ hepatorenal syndrome (HRS) $+ 1.2069 \times$ LC $- 1.1555 \times$ hepatitis B e antigen (HBeAg) $- 0.1003 \times$ ALB $- 0.042 \times$ PTA.

That scoring system has been used in several centers for liver diseases, and its effectiveness and accuracy have now been confirmed in these centers.

In summary, to establish a new scoring system on the basis of China's national status of liver disease and to further validate this system, large multicentered studies with a large number of patients is crucial before they are adopted in China. It is important that any new system developed under these conditions be subjected to rigorous study, standardization and scientific validation.

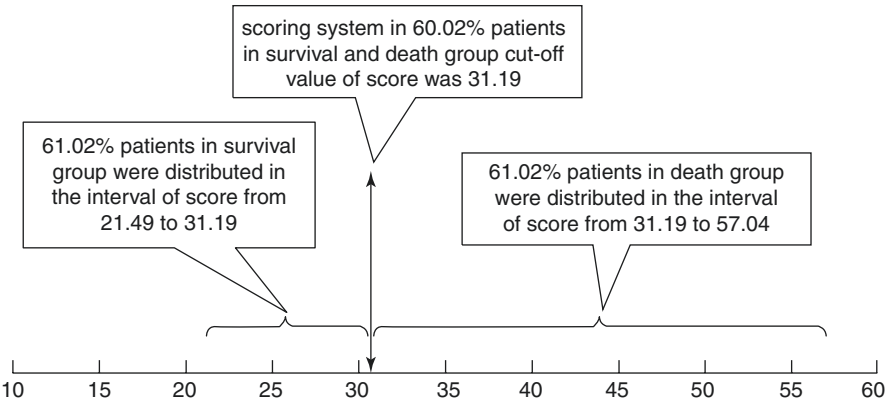

Fig. 1.8 Cut-off value and distribution range of total score determined with the MELD scoring system in 60.02% of surviving and deceased patients

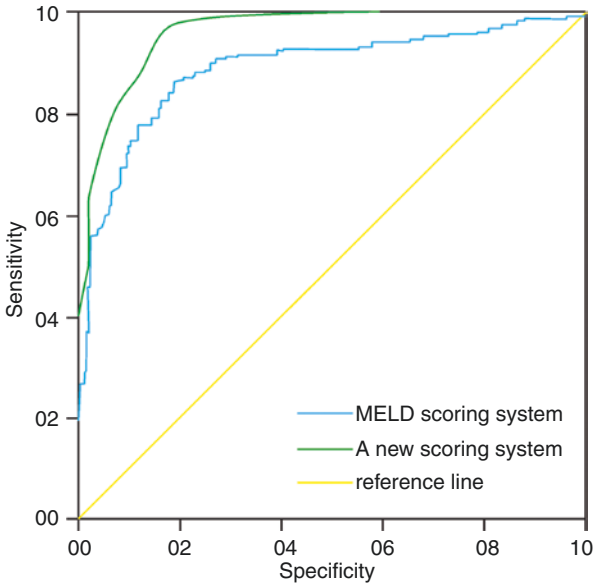

Fig. 1.9 Receiver operator curve of a new scoring system and MELD scoring system

1.6.4 Perioperative Therapeutic Regimens Following Liver Transplantation in Hepatitis B-Induced Liver Failure Patients

For liver transplantations in patients with hepatitis B-induced liver failure, perioperative therapy is crucial, impacting the recurrence of hepatitis B virus reinfection following liver transplantation. In fact, perioperative therapy can make a major

difference in patients with hepatitis B compared to patients with other liver diseases (e.g., tumor recurrence). It is well appreciated that antiviral therapy is necessary in hepatitis B virus patients as discussed below.

1.6.4.1 Antiviral Therapy During the Perioperative Period

The Guideline for the Prevention and Treatment of Chronic Hepatitis B in China (2010) recommends that (1) for patients with hepatitis B planning to receive liver transplantation, oral lamivudine be administered within 1–3 months before liver transplantation when hepatitis B virus DNA is detectable (100 mg *q* 24 h). Hepatitis B immune globulin (HBIG) should be administered during the anhepatic stage in surgery and long-term use of lamivudine and low-dose HBIG (800 IU *q* 24 h for the first week postsurgical, 800 IU *q* 1 week, then 800 IU *q* 1 month). The dose of HBIG and interval between two treatments [generally, trough hepatitis B surface antibodies (anti-HBs) are >100–150 mIU/mL and anti-HBs is better if >500 mIU/mL within 6 months of surgery) should be determined according to the anti-HB levels. For patients nonresponsive to lamivudine, other nucleos(t)ide analogues effective for resistance mutation should be used and for patients with a low risk for recurrence of HBV infection (i.e., HBV DNA negative before liver transplantation and absence of HBV infection recurrence within 2 years after liver transplantation), lamivudine plus adefovir should be considered for the prevention of HBV infection recurrence.

1.6.4.2 Therapy with Artificial Liver Support System During the Perioperative Period

While patients are waiting for liver transplantation, an artificial liver support system (ALSS) may be of use as a bridge to transplantation. Liver failure may significantly compromise the detoxication, synthesis, and metabolism activities, resulting in a significant accumulation of toxins and deficiency of some important factors (such as coagulation factors). Unfortunately, some patients die while waiting for either their liver function to improve or for liver transplantation. ALSS have been generally used to partially substitute the liver's normal activities, such as clearing some toxins and supplement some missing factors, which is helpful for life maintenance while awaiting subsequent liver transplantation or spontaneous recovery. Thus, for patients with liver failure secondary to hepatitis B, ALSS maybe an important therapeutic strategy while patients are waiting for liver transplantation.

ALSS can be classified as a mechanical artificial liver, biological artificial liver, or mixed artificial liver. Mechanical ALSSs mainly utilizes nonbiological materials to clear toxins in the body and supplement some missing factors. Biological ALSSs use biological materials to substitute the liver's activities. The core feature of the biological ALSS systems is to simulate hepatocyte function. However, the source and biosafety, as well as their location (bioreactor), are important factors limiting the development of biological ALSSs. Finally, mixed ALSSs employ the hemodiafiltration, plasma exchange, and hemoperfusion that are able to detoxify substances (nonbiological ALSS) and human or porcine "hepatocytes" in the bioreactor.

Currently, nonbiological ALSSs are the most widely used in clinical practice, and biological and mixed ALSSs are still undergoing investigation and have not been widely applied in clinical practice.

In addition to its use before liver transplantation, ALSS post liver transplantation may be used to correct ongoing renal failure, brain edema, severe water and electrolyte disturbances, and severe infections.

Another factor to consider is that liver donors are rare. As a result, the inability to perform liver transplantation in a timely fashion results in reduced survival rates. Under those conditions, pre transplant complications including multi organ failure may persist. In such cases, ALSS therapy may prove to be beneficial.

1.6.4.3 Other Managements and Therapies During the Perioperative Period

For patients with hepatitis B-related liver failure, perioperative therapies are more important than in patients receiving elective liver transplantation (such as liver tumor patients without liver failure). Liver failure causes a variety of complications that directly contribute to transplantation failure and a high-risk status after surgery. Clinicians need to address problems such as portal hypertension, upper gastrointestinal bleeding, severe jaundice, ascites, spontaneous peritonitis, hepatic encephalopathy, hepatopulmonary syndrome, portal pulmonary hypertension, kidney dysfunction, hepatorenal syndrome (among others) through active treatment/management in specialized liver units. Preserving liver function and maintaining a normal physiological status have been shown to reduce the risk of therapeutic failure in patients receiving liver transplantation due to hepatitis B-related liver failure.

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