6 Prognosis, Prevention and Research Prospects of Progression to Severe Hepatitis B (Liver Failure)

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Abstract

This chapter describes the factors involved in the disease prognosis, parameters of outcome evaluations, principles and techniques for progression prevention. In last section, the future perspectives in both basic and clinical investigations towards unmet medical needs in AECHB and HBV ACLF are discussed.

1. Factors affecting the prognosis of patients with severe hepatitis B include those related to the virus (including viral load, HBeAg expression, and gene mutation), patient age, co-morbidity, TBil, INR, serum Cr, and the host genetic background. Indicators associated with patient prognosis include TBil, total cholesterol, albumin and prealbumin, hepatic encephalopathy, kidney damage, alpha-fetoprotein and vitamin D binding protein, blood sodium level, virus HBeAg expression and genotype, and blood glucose.
2. In addition to TBil, INR, hepatic encephalopathy, Cr level and AFP as indicators for prognosis of severe hepatitis, some other parameters such as clinical signs, symptoms, serum levels of total cholesterol and albumin and natrium, and coagulation factors are all valuable in assessment. The roles of cell apoptosis, liver regeneration and immunological parameters in assessing patient prognosis are

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under study. Prognostic evaluating systems include MELD score, MELD-Na score, iMELD score, KCI and CTP score.
3. Prevention of severe hepatitis B should be started in asymptomatic patients. Close observation, sufficient rest, adequate nutrition, meticulous nursing and psychological care, preventing and removing exacerbating factors, treating concomitant diseases, reasonable antiviral and comprehensive therapies are helpful to prevent CHB patients from developing to severe hepatitis. For patients who already have severe hepatitis B, the prevention and management of complications is important for lowering mortality rate.
4. New research directions in acute-on-chronic liver failure include: (1) Additional well controlled studies using present or new liver systems are warranted. Other approaches include the use of granulocyte colony stimulating factor to treat infections as well as the potential of use of stem cells to restore immune integrity and enhance liver regeneration. (2) Using new cell lines and animal models to understand the molecular biology of HBV, the immune response and to develop novel therapies. (3) Development of new anti-HBV strategies, e.g. silencing or remove cccDNA, enhancing immunologic clearance of HBV infection, inhibiting virus entry or HBc expression and using CRISP to disrupt cccDNA.

6.1 Prognosis of Severe Hepatitis B

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Hepatitis B virus (HBV) related severe hepatitis is a well recognized entity in the world but especially relevant to Asian countries including China. It can be divided into four types, acute, sub-acute, acute on-chronic and chronic, according to the Guidelines of Liver Failure published in 2006 [1]. HBV is known to be the most common cause of liver failure in Asia. Severe hepatitis has a high morbidity and mortality and is characterized by a prolonged prothrombin time, elevated total bilirubin and the presence of hepatic encephalopathy (HE). Chronic severe hepatitis (CSH) occurs when there is massive hepatic necrosis in patients who already have chronic hepatitis. The prognosis is related primarily to the amount of hepatic injury and to the degree of hepatocyte regeneration. The incidence of CSH is rising especially in patients with acute on chronic liver failure (ACLF) and chronic liver failure (CLF) and with established cirrhosis. Here we discuss the factors that influence outcome in patients who develop CSH.

6.1.1 Factors Influencing Prognosis of Severe Hepatitis B

The prognosis is primarily determined by a number of important factors. First, the etiology is the most important factor affecting outcome according to AASLD guidelines (Treatment of acute liver failure of AASLD 2005 and 2011). In China, the

most common cause is HBV infection and it is reported that viral load (HBV DNA) and viral variation (HBeAg status) are the best indicators of prognosis. However, clinical status including degree of jaundice and presence of hepatic encephalopathy (HE) and poor coagulation as measured by INR and presence of renal failure are all associated with poor outcome. However, it is well recognized that even the presence of these factors is not always associated with poor outcome. The AASLD has now well established criteria of poor outcome for patients who develop acute liver failure (ALF). These include prolongation of the international normalized ratio (INR) to >1.5 and the presence of hepatic encephalopathy (HE). In China, where acute on chronic disease is more prevalent the presence of HE is known to be associated with a poor outcome. However, although HE is important both for or diagnosis and prognosis of CSH, HE is not a common finding in CSH in China and subclinical HE is difficult to diagnose. In North America and Europe, HE is easily diagnosed in patients with acute liver injury, whereas while in China jaundice, not HE, was common in patients with CSH. Newer models that have been adapted to assess severity of liver disease including the Mayo Model for end stage liver disease (MELD) have not proven to be of value in patients with CSH.

In addition to etiology, a number of other factors influence outcome of CSH, including age, co-morbidities, total bilirubin level, prothrombin time (INR), and serum creatinine. Other additional factors that may be of value include the measurement of levels of α fetal protein (AFP) which may be indicative of hepatic regeneration and complications including the development of HE and hepato-renal syndrome.

6.1.1.1 Age

Patients over 40 years of age who develop ALF and CSH have a higher mortality. Although the exact reason for this is not clear it is known that elderly patients have poor immunity and often succumb to severe infections. Additionally, hepatocyte regeneration is reduced in the elderly.

6.1.1.2 Host Factors

There have been few studies on genetic susceptibility to severe hepatitis B. However, data is emerging largely derived from studies conducted in the Asian population that variability in both innate and adaptive immune response genes including tumor necrosis factor (TNF-α and TNF-β), interleukin-10 (IL-10), interferon-γ inducible protein 10 (IP-10, CXCL-10), vitamin D receptor (VDR), and human leukocyte antigen (HLA) as well as the ability to mount an appropriate cell mediated antiviral response are associated with outcome.

6.1.2 Prognostic Indicators of Severe Hepatitis B

6.1.2.1 Total Bilirubin

Bilirubin is taken up and metabolized in the liver, so that levels of serum bilirubin are an excellent measure of liver excretory function. High bilirubin levels are indicative of deterioration of liver function. In CSH there is massive hepatocyte necrosis,

which results in decreased absorption, transportation, binding and excretion of bilirubin. Thus, measurement of serum total bilirubin level is positively correlate with degree of hepatocyte necrosis. Furthermore in CSH, it is known that patients with high bilirubin level have a poor prognosis.

6.1.2.2 Serum Cholesterol

The liver also plays a key role in the absorption, synthesis and transportation of cholesterol. Liver supplies 60–80% total body cholesterol. When hepatocytes are severely damaged, the synthesis of cholesterol decreases and cholesterol levels correlate with degree of liver injury and mortality. Thus, levels of cholesterol are indicator of poor liver synthetic function.

6.1.2.3 Serum Albumin

Albumin is known to be synthesized in the rough endoplasmic reticulum (RER) of hepatocytes. When hepatocytes are severely damaged, synthesis of albumin decreases and serum albumin levels fall. However as the half life of albumin is 20 days, measurements of albumin do not reflect acute injury and therefore do not correlate with prognosis especially in patients with acute liver necrosis. Furthermore, the use of albumin infusions also limits the usefulness of measurement of serum albumin as a predictor of outcome. Prealbumin is also synthesized by hepatocytes and has a shorter half-life. It is relatively easy and inexpensive to measure prealbumin and thus measurement of serum prealbumin levels potentially could be a more sensitive and specific measure of liver function.

6.1.2.4 Hepatic Encephalopathy (HE)

HE is neurological and psychiatric disorder that is seen during all phases of liver failure. But especially in patients with CSH and cirrhosis. Precipitating causes of HE include upper gastrointestinal tract hemorrhage, ingestion of a high protein diet, infection and large volume drainage of ascites. It has been reported by the King's College Group that the presence of HE and a high INR are associated with poor outcome in ALF (ref). It is also known that patients with CSH who develop HE also have a poor prognosis and high mortality. Thus it is important to prevent and early treat HE to improve patient outcomes.

6.1.2.5 Acute Kidney Injury (AKI) and HRS

Disturbances in hemodynamic parameters including systemic vasodilation is a common complication of cirrhosis. The result of changes in blood flow in the splanchnic system leads to reduced blood flow to abdominal organs including the liver and kidneys. This leads to water sodium retention (ascites formation), increased total intravascular volume and increased cardiac output. The development of liver cirrhosis, leads to systemic vasoconstriction and reduced renal blood flow. The compensatory increase in cardiac output is usually insufficient to sustain perfusion pressure (high output heart failure), leading to a further reduction in renal blood flow and kidney failure. Ultimately this may lead to the development of hepatorenal syndrome (HRS), which is one of the most serious complications of end-stage liver disease.

As a consequence of complications of cirrhosis including GI hemorrhage, ascites, serious infections, patients often develop acute renal injury manifested by oliguria or anuria, azotemia and electrolyte imbalance. Although the pathogenesis of HRS is not totally known, most scholars believe that it is secondary to a functional rather than structural renal change. Once HRS develops survival rates decrease markedly and patients survival may only be 1–3 months. Differences between AKI and HRS were as following: (1) HRS occurs in patients with liver cirrhosis who have ascites and a serum creatinine (Cr) >133 μmol/L; (2) acute kidney injury (AKI) is characterized by a precipitous rise in serum creatinine Cr to >26.4 mmol/L, or a 50% increase above baseline. To firmly make the diagnosis of HRS it is important to rule out extra hepatic causes of renal failure. In contrast the diagnosis of AKI is relatively straightforward but treatment must be individualized and directed to precipitating causes.

6.1.2.6 AFP

Serum alfa fetoprotein (AFP) is a glycoprotein which is mainly synthetized in human embryonic liver. Levels of AFP decrease with age but increases can be seen in patients with acute or chronic viral hepatitis reflecting both liver damage and liver cell regeneration. Levels of AFP in patients with fulminant hepatitis, is regarded as an index of liver cell regeneration, AFP decreases in patients with severe hepatitis indicating stem cell necrosis and poor regeneration. In a prospective study, AFP levels in ALF had a dynamic change; a higher level of AFP was not found to be associated with a good prognosis, however, patients with high levels of AFP levels within 3 days after hospitalization had a better prognosis, probably reflecting hepatocyte regeneration. In patients with CSH, levels of AFP remained above baseline probably reflecting ongoing liver damage. Sustained elevations of AFP are often associated with the development of hepatocellular carcinoma (HCC).

6.1.2.7 Gc Protein

Gc protein, also referred to Vitamin D-binding protein (DBP), is an α globulin and a member of the multi-gene superfamily which includes albumin, prealbumin and alpha fetal protein (AFP). Most of these proteins have approximately a 55 kU in molecular mass and are secreted by the hepatic parenchymal cells. Gc protein is highly abundant in serum and binds to vitamin D and has many physiological functions, including the removal of actin, enhancement of chemotactic activity and macrophage activation of C5 on neutrophils, regulation of the activity of osteoclasts and transport of fatty acid and endotoxin. As Gc protein is produced by liver cells, changes in levels of Gc protein can reflect changes in liver function including the amount of hepatic necrosis and degree of liver reserve. Thus measurements of levels of Gc may be if use in estimating the prognosis of patients with fulminant hepatic necrosis. It has been suggested that reduced levels of Gc protein may lead to actin deposition in the blood vessels leading to multiple organ failure. Therefore, Gc protein can also be a useful indicator for measuring ALF multiple organ failure.

6.1.2.8 Serum Sodium Level

Hyponatremia is seen often in patients with CSH. Serum sodium concentration in patients is related with presence of liver cirrhosis, refractory ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and hepatorenal syndrome [2]. Some believe that hyponatremia should be added into the new model for end-stage liver disease (Na MELD) to improve its predictability (ref). A number of studies have suggested that hyponatremia is an important index for evaluating the prognosis of liver cirrhosis. Studies have shown that relative risk (RR) of levels of serum sodium was 0.023, which suggest that serum sodium may be a protective factor affecting the prognosis. So, maintaining normal serum sodium could reduce the risk of death in patients with severe hepatitis. Hyponatremia might result in increase of osmotic pressure difference between intracellular and extracellular pressure and induce or aggravate brain edema and increase the incidence of hepatic encephalopathy and central pons demyelinating lesions. Central pons demyelinating lesions with hyponatremia might be precipitated by rapidly replacing sodium. It was worth noting that although hyponatremia, refractory ascites and AKI can all exist independently, but in most patients they are interconnected (Fig. 6.1). Hyponatremia is seen in nearly half of patients with decompensated cirrhosis who have sodium and water retention. Hyponatremia is known to be associated with intractable ascites and AKI. Once refractory ascites develops, worsening hyponatremia and sodium and water retention results in AKI. Finally, AKI, as a final complication, in turn, leads to hyponatremia (including water sodium retention) and refractory ascites. Given the extremely high case fatality rate for AKI, dealing with hyponatremia as soon as it develops is key to prevent refractory ascites and AKI. Therefore, the prevention of hyponatremia might improve the survival rate in patients with CSH.

Hyponatremia and water-sodium retention can become important reasons of refractory ascites and AKI, meanwhile, refractory ascites can also lead to further development of hyponatremia and water-sodium retention, and result in the incidence of the complication of AKI eventually; Following the incidence of AKI, it will in turn cause the occurrence of hyponatremia (including water-sodium retention) and refractory ascites.

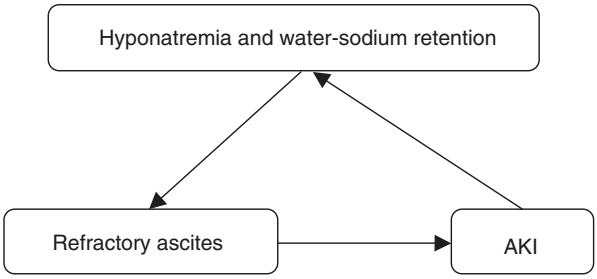

Fig. 6.1 The relationship among the hyponatremia, refractory ascites and AKI

6.1.2.9 Virus Factors

In Asia, serious hepatitis B occurs mostly on the basis of CHB, CHB is divided into two categories: HBeAg positive and HBeAg negative, the pre-C area or core area promoter mutation of hepatitis B virus (HBV) is the main mechanism of severe HBeAg-negative CHB development. At present, reports from China as well as the rest of the world show that the proportion of HBeAg negative CHB patients is increasing. As an example data emerging from Taiwan and Hong Kong shows that the proportion of HBeAg negative CHB is about 90% of total CHB [3]. The effect of HBeAg on the prognosis of severe hepatitis has now been studied extensively, and it was found that the prognosis of HBeAg negative severe hepatitis B patients was worse than that of HBeAg positive patients. A possible reason for this finding is that chronically infected patients are HBeAg negative for a long time, their average age is greater and these patients have liver cirrhosis. The presence of liver cirrhosis is an important prognostic factor in patients with CSH. It is known that, patients with cirrhosis are more susceptible to hepatic decompensation, the development of hepatic encephalopathy and hepatorenal syndrome.

6.1.2.10 Genotype

Studies in China and throughout the world have shown that HBV genotype may also be related to disease progression, clinical manifestations, prognosis and response to antiviral therapy. Although a number of studies have examined the effect of genotype on anti-HBV response and drug resistance, there has been to date no systematic and comprehensive study examining this and the published results differ significantly. A number of studies have examined the effect of interferon therapy on HBeAg positive CHB patients. Hou et al. showed, that the treatment response of patients who were infected with HBV genotype A was better than that of patients who were infected with non-genotype A [4]. Kao et al. from Taiwan reported in their study that the response rate was 41% and 15% in 58 cases of patients with CHB and chronic hepatitis C (CHC), respectively [5]. There is still considerable debate regarding the inconsistent research results in China investigating the effect of lamivudine on anti-HBV responses. Studies have shown that the treatment response and resistance rate of genotype B were better than those of genotype C, the HBV YMDD mutant (YIDD/YVDD) occurred frequently in genotype C and genotype D. Long-term efficacy of lamivudine on anti-HBV responses conducted by Kobayashi et al. showed that genotype A was more susceptible to develop the YMDD mutation than genotype B and genotype C.

HBV DNA levels have been found to affect the prognosis of patients with chronic severe hepatitis B. A previous study reported that the HBV DNA level in patients who died was higher than that in patients who responded to medical therapy (ref). In the group of patients with chronic severe hepatitis B who died, HBV DNA high replication accounted for 61.26% of the total cohort. Although the virus load was not directly related to liver damage in patients with chronic severe hepatitis, HBV DNA negative conversion was an important factor that disease could be controlled. Hache et al. also has reported that serum HBV DNA levels were independent predictors of disease progression of hepatitis B [6]. In that study, the rates of

improvement and median survival time of 3 months in patients with early and late treatment with antiviral therapy, suggesting that use of early antiviral therapy can reduce hepatic necrosis and promote tissue repair and regeneration through reduction of HBV replication.

6.1.2.11 Fasting Blood Glucose (FBG)

The liver is known to be important for maintaining blood sugar balance through glycogen synthesis and gluconeogenesis. Patients with severe hepatitis have a decreased ability to inactivate insulin, leading to increased blood insulin levels and low blood sugar levels. Fasting blood glucose levels were closely related to the severity of hepatitis and in patients with severe hepatic necrosis the incidence of hypoglycemia has been reported to be higher. Fasting blood glucose levels are negatively correlated with serum total Bilirubin and positively correlated with PTA value. At the same time, fasting blood glucose levels were closely related to prognosis. The presence of hypoglycemia is the setting of CSH is associated with a lower clinical recovery rate and worse prognosis. These results fully showed that detection of fasting blood glucose is important to understand the degree of liver cell necrosis, and its monitoring may be useful in predicting the survival rates in patients with CSH.

6.1.3 Indicators for Evaluating Prognosis of Severe Hepatitis B

A Diagnostic model of prevention and control standards for patients with CSH was created at the 2000 national conference on the 10th of viral hepatitis in Xi'an. In 2006, experts from China came together to establish guidelines for the diagnosis, prognosis and treatment of patients with liver failure. Meta analysis of patients with CSH (2429 cases) found that total bilirubin, prothrombin activity (PTA), hepatic encephalopathy, levels of serum creatinine and AFP were different between patients who survived and died (Table 6.1).

Table 6.1 Analysis of the comparison results of TBil, PTA, AFP, hepatic encephalopathy and creatinine level between the survival group and the death group

Variable	Number of research literature	Heterogeneity			Effect sizes and parameters			
		Q	*P*	I^2%	WMD	95% CI	Z	*P*
T.BiL	14	155.91	0.00	89.5	132.10	96.40–177.90	5.66	0.00
PTA	9	93.62	0.00	54.3	−7.75	−10.20 to − 5.20	6.03	0.00
AFP	6	75.20	0.00	92.0	−76.90	−129.29 to − 24.50	2.88	0.01
Creatinine	5	6.17	0.29	35.1	0.39	0.27–0.51	6.61	0.00
HE	7	14.96	0.04	62.1	1.38	1.27–1.49	8.02	0.00

Redraw from Lin Ju-Sheng, et al. Meta analysis on prognostic factors of patients with severe hepatitis B. Chinese Journal of Experimental and Clinical Infectious Diseases (Electronic Edition): 2011. 5(1): 14–19. https://doi.org/10.3877/cma.j.issn.1674-1358.2011.01.003 [Article in Chinese] [7]

In recent years, studies have shown that some clinical signs of liver failure patients, serum biochemical/radiographic parameters and application of antiviral therapy were closely related to the prognosis of CSH suggesting that these data could be used for assessing the prognosis of liver failure.

6.1.3.1 Clinical Signs

It is known that a number of clinical parameters are associated with outcome to HBV infection including male gender, older age, poor nutritional status, development of jaundice and signs of clinical deterioration including presence of ascites or poor coagulation (bruising). Although not specific, they are associated with poor outcomes.

6.1.3.2 Biochemical Parameters

Besides routine laboratory tests including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST/ALT ratio, α fetal protein (AFP), total bilirubin (TBil), prothrombin activity (PTA), total cholesterol (TC), cholinesterase (CHE) and albumin, in recent years a number of factors related to the prognosis have been found. These include arterial blood ketone body ratio, imaging examination, levels of the Gc protein, osteopontin, fibronectin, cortisol concentration, serum free fatty acids, prealbumin, fasting glucose, tumor necrosis factor alpha (TNFα), c-reactive protein (CRP), peripheral blood vitamin D binding protein, serum sodium, arterial blood lactic acid salt and blood ammonia [8–10].

6.1.3.3 Prognostic Indicators Still in the Research

Some additional markers might be associated with the prognosis of CSH, including hepatocellular apoptosis or necrosis related markers (coagulation protein sol, M65/antigen, cytochrome C, etc.), liver cell proliferation, regeneration markers such as follicle inhibition/activin A (follistatin/activin A, F/A) ratio, aging marker protein 30 (senescence marker protein 30, SMP30), stem cell factor (stem cell factor, SCF), and factors that promote the platelets (TPO thrombopoietin). It is well known that the immune system contributes both to liver injury and repair. For example, HBV infected hepatocytes are surrounded by a large number of mononuclear—macrophage cells (Kupffer cells), which contribute to liver cell apoptosis through secretion and release of a number of inflammatory cytokines including TNF alpha, interleukins and interferons. Some indicators reflect mono-macrophage function including expression of CD163, CD154, human leukocyte antigen (human leukocyte antigen, HLA) and other biomarkers such as micro RNAs (miRNAs), called S100b, troponin I and other markers, which also showed potential prognostic value of CSH.

6.1.3.4 Coagulation Factors

The liver is pivotal to coagulation and the vast majority of clotting factors such as I, II, VII, X, XI are synthesized in the liver. Patients with CSH, who have marked liver parenchyma necrosis have reduced hepatic synthesis of clotting factors, increased consumption leading to prolongation of prothrombin time and PTA decline.

Table 6.2 Biological half-lives of liver-synthesized clotting factors

Clotting factors	Half-life
Factor I (Fibrinogen)	1.5–6.3 days
Factor II (Prothrombin)	2.8–4.4 days[a]
Factor V	12–36 h
Factor VII	2–5 h[a]
Factor IX	20–52 h[a]
Factor X	32–48 h[a]

[a]Vitamin K-dependent posttranslational carboxylation

Fulminant hepatitis is associated with marked disturbances in coagulation including a decrease a number of clotting factors, especially factor VII and V (Table 6.2). Measurement of prothrombin time (PT) and activated partial-thromboplastin time (APTT) is used to evaluate the risk of bleeding in ALF patients. Used international standardization ratio (INR) to measure PT, when INR 1.5 or higher, is an important diagnostic and prognostic indicator of ALF. When the INR ≥6.5 prognosis was generally poor. Researchers have shown that through comprehensive Logistic regression analysis older age (>40 years), plasma prothrombin activity decrease (≤10%) and the presence of systemic inflammatory response syndrome (SIRS) are indicators of poor prognosis of CSH.

Coagulation dysfunction is an important diagnostic and prognostic indicator for liver failure (including CSH), so monitoring of coagulation factors has proven to be a useful indicator of poor prognosis Platelet was one of important participators in coagulation. Numbers and function of blood platelets change during liver dysfunction. Because thrombopoietin (TPO) is synthesized primarily in the liver, when CSH occurred, TPO synthesis decreases, resulting in decreased numbers of platelets. Chan et al. has reported that only platelet count (≤143*E+09/L) and serum bilirubin (>172 μmol/L) are independent prognostic factors for mortality of CSH in patients without HE. Mortality of CSH with high blood bilirubin and a low platelet count was 69%; with a low platelet count alone was 11; with a high blood bilirubin was 13% and without both there was no mortality. Others have confirmed these results (Table 6.3).

6.1.4 Prognosis Assessment Analysis of Severe Hepatitis B (Comparison of Different Analysis System)

CSH accompanied by marked liver cell necrosis caused by excessive immune response is associated with a very high mortality. According to the severity of disease it is critical to evaluate the early prognosis to guide the best clinical treatment, including comprehensive medical treatment, artificial liver support and liver transplantation. Different countries have now adopted MELD and the King's College scoring system to evaluate CSH. The MELD score was mainly used initially to determine candidacy for TIPS and later adapted to determine priority of liver transplantation [11]. Factors affecting prognosis of CSH have always been hot spots of

Table 6.3 The independent prognostic factors of acute-on-chronic severe hepatitis B

	Chan et al. N = 46	Yuen et al. N = 47	Chien et al. N = 91	Tsubota et al. N = 50
Age	–	–	–	–
Sex	–	–	–	–
Albumin	–	Reduce	–	–
Bilirubin	Increase (>172)	Increase	Increase	Increase
Prothrombin time	–	Extend	Extend	Extend
ALT	–	–	–	–
PLT	Reduce ($\leq 143 \times 10^9$/L)	Reduce	–	–
HBeAg	–	–	–	–
HBV DNA	No study	–	–	–
Serum creatinine	–	–	–	No study
AFP	No study	–	–	–
Liver fibrosis	No study	Emergence	–	Emergence
Ascites	No study	–	Emergence	–
Child–Pugh grade	No study	High	High	No study

HBeAg Hepatitis b virus e antigen, – no difference

research. Global regional different etiology of CSH causes different prognostic factors, and ALF internationally is mainly caused by the overuse of acetaminophen, but by HBV infection in China.

6.1.4.1 MELD Scoring System

The model for end-stage liver diseases (MELD) mainly used bilirubin, INR and Creatinine and etiology to evaluate end-stage liver disease. In 2000 Malinchoc et al. first used MELD to predict mortality of end-stage liver disease patients received transjugular intrahepatic portosystemic shunt (TIPS) and confirmed that MELD was useful to predict end-stage liver disease mortality and postoperative survival time [12]. The MELD formula was, R = 3.8 ln[total bilirubin (mg/dL)] + 11.2 × ln(INR) + 9.6 ln/creatinine (mg/dL) + 6.4 × (etiology: cholestasis and alcoholic liver cirrhosis is 0, other reasons for 1). Kamath et al. modified formula for convenient for calculating as follows: R = 3.8 ln[total bilirubin (mg/dL)] + 11.2 ln(INR) + 9.6 ln/creatinine (mg/dL) + 6.4 (etiology: bile or alcoholic 0, other 1). Value of R positively correlated with mortality and negatively correlated with survival rate [13].

The MELD score for patients with liver failure provided an effective evaluation index. In MELD classification, however, serum creatinine, bilirubin and INR, were easily influenced by other diseases, which could directly affect liver disease. Therefore, in order to avoid extrahepatic factors fluctuations affecting accuracy of the MELD classification of serum creatinine, MELD classification should be used in the patient's hemodynamic stability and on the basis of full rehydration. At the same time, MELD score had yet to incorporate other factors which affect outcome including, ascites, hemorrhage, hepatic encephalopathy, because complications of liver cirrhosis including the presence of portal hypertension might directly threaten

patients' life. The MELD scoring system has proven to be a highly effective model for assessing end-stage liver disease, and had important clinical value in evaluating liver disease severity and prognosis.

6.1.4.2 MELD-Na Scoring System

Hyponatremia in liver failure patients is mainly caused by peripheral arterial expansion, effective blood volume reduction, peripheral blood circulation deficiency stimulating pressure sensor and volume receptor activating three kinds of vasoconstrictor systems, including the vasopressin system, the aldosterone renin-vasoconstriction system and the sympathetic nervous system, all of which act on kidneys, causing water sodium retention and diluted hyponatremia. These neurohumoral systems and the degree of kidney damage are associated with the severity of portal hypertension. Thus, serum sodium could be used to reflect the severity of portal hypertension. Portal hypertension is an important factor in patients with liver failure leading to a number of complications and is a leading cause of death in CSH. Some reports have shown that hyponatremia is an independent predictor of hepatorenal syndrome, and it has become apparent that serum sodium is an important prognosticator of liver cirrhosis. Recognition of this has led to adding serum sodium into MELD to define new scoring formula. The MELD-Na calculation formula is: the MELD score-Na = MELD + 1.59 × [135 − serum sodium (mmol/L)]. When serum sodium is greater than 135 mmol/L, serum sodium is equal to 135 mmol/L in calculation. When serum sodium is less than 120 mmol/L, serum sodium is equal to 120 mmol/L in calculation. Studies have now confirmed that MELD-Na could predict prognosis of patients with end-stage more precisely than MELD, but there was no significant statistical difference. It has been suggested that larger studies will confirm the value of adding Na to MELD in assessing patient mortality.

The MELD-Na score can effectively predict the 3 month mortality rates of patients with CSH, and also can be used as a therapeutic evaluation index. Some researches using MELD score combined with CPT have shown that MELD ≥18 and TC ≤2.8 nmol/L could be used to predict the long-term prognosis of decompensated cirrhosis, but its predictive value still has not been studied in liver failure (including CSH). Li et al. investigated 213 patients (213 cases of CSH) and found that rates of cirrhosis, infection, hepatic encephalopathy (HE) level, total bilirubin, total cholesterol (CHO), cholinesterase (CHE), blood urea nitrogen (BUN), serum creatinine (Cr), serum sodium, white blood cell count (WBC), α fetus protein (AFP), international standardization ratio (INR) and PT were different between patients who died and those who survived, However there was no difference in ALT, AST, albumin and hemoglobin (HGB) between the two groups. The study finally developed a regression model using multivariate analysis, as follows: Logit (P) = 1.573 * Age + 1.338 * HE − 0.011 * CHO + 0.011 * 1.608 Cr − 0.109 * Na + 1.298 * INR + 11.057. Data showed that this model had a higher predictive value (e.g., introduction of age) than MELD, but its application in clinical practice needs further investigation for validation.

6.1.4.3 iMELD Scoring System

Integrated Model for End-stage Liver Disease (iMELD) Score was established by Luca et al. It adds age and serum sodium to MELD and the formula is as follow: iMELD = MELD + 0.3 × (age) − (0.7 × serum sodium) + 100. Studies have shown that adding age and serum sodium into the original MELD formula could strengthen its survival prediction.

The MELD, MELD-Na and iMELD scoring systems do not include any complications in their formulation. In a large number of cases, however, it has been shown that complications of liver disease lead to deterioration and shorten survival time. Additionally, in China the majority of patients with liver failure are secondary to HBV infection, and studies have demonstrated that antiviral treatment is an independent predictor for prognosis of liver failure. Therefore, in clinical practice the use of a multi-factor MELD prognostic index regression model should be adopted to predict prognosis of liver failure first, and then the use of MELD-Na and iMELD in combination with relevant clinical issues including complications, and the use of antiviral treatment may improve the predictability of MELD to make more accurate judgment to prognosis.

6.1.4.4 King's College Criteria

The King's College Criteria has been widely adopted worldwide to assess patients with acute liver failure (ref). Although its specificity is high, its sensitivity is not high. And it has not proven to be as useful for patients with subacute liver failure and CSH.

6.1.4.5 Child-Turcotte-Pugh (CTP) Scoring

The CTP scoring has been used to quantify the liver reserve function of patients with established cirrhosis. CT scoring was first put forward by Child and Turcotte, then Pugh replaced general condition with hepatic encephalopathy and the scoring system was renamed CTP. In CTP, the score ranges from 5 to 15. Liver reserve is divided into A, B, C level 3 (Table 6.4). The CTP scoring system is mainly used for patients with liver cirrhosis. As CSH is most commonly seen in patients with cirrhosis, the use of the CTP scoring system may be useful for clinical diagnosis, treatment and prognosis. However, CTP also has its shortcomings, (1) the main target population was people with cirrhosis; (2) it includes subjective parameters, i.e. degrees of ascites and hepatic encephalopathy, so it is difficult to standardize these

Table 6.4 CTP scoring criteria

Indicator	1 point	2 points	3 points
HE (grade)	No	1–2	3–4
Ascites	No	Mild	Medium and severe
TBil (μmol/L)	<34	34–51	>51
Albumin (g/L)	>35	28–35	<28
Prothrombin time extended (s)	<4	4–6	>6

parameters; (3) CTP could not differentiate anomalies and significance of abnormal laboratory parameters, known as the upper limit effect (ceiling effect); (4) objective indicators such as albumin and PTA was not standardized across laboratories; (5) the scores were too concentrated. There are only 8 points between maximum and minimum, and thus many patients had same score. Despite these limitations, the sensitivity of CTP scoring predicting mortality was about 78%, and specificity was 83% [14]. However the CTP scoring system has not proven to be as useful to assess patients with CSH. However, in China as the main cause of CSH is on the basis of chronic HBV infection and most of the patients had cirrhosis, the CTP scoring system has proven to be more useful for evaluating liver function reserve and furthermore it has proven easy to use. In the clinic, using a combination of MELD and CTP to assess liver function and prognosis has proven to be useful.

6.1.5 Lethal Factors of Severe Hepatitis B

CSH caused by HBV produces a syndrome similar to acute liver failure which is characterized by severe clinical symptoms and high mortality. As the liver is central to maintaining normal human physiology, damage to the liver affects multiple systems including the lung, heart, kidney and coagulation systems often leading to death. Therefore, in an attempt to improve the survival rate and prognosis of patients, research is now focused on the multi system alterations that occur in patients with CSH.

6.1.5.1 Bacterial Infections and Systemic Inflammatory Response Syndrome

Bacterial infection is a common complication of liver failure, especially chronic liver failure with decompensated cirrhosis. An acute infection in patients with established cirrhosis often leads to further patient deterioration. Patients with cirrhosis are prone to bacterial infection because of an increase in bacterial translocation and reduced mononuclear/macrophage function [15, 16]. The presence of ascites in CSH is known to be associated with an increased risk of spontaneous bacterial peritonitis (SBP). As a consequence of SBP, there is a release of pro-inflammatory mediators including TNF, IFN gamma and nitric oxide synthase (NOs) which lead to activation of the coagulation system (thrombosis), reduced systemic vascular resistance leading to hypotension. Ultimately the patient may present in a shock like state with high levels of serum lactate reflecting tissue hypoperfusion. It is now known that bacterial infection is one of the most important factors leading to death in CSH.

6.1.5.2 Upper Gastrointestinal Hemorrhage and Hepatic Encephalopathy

Upper gastrointestinal hemorrhage is a common complication of cirrhosis reflecting the presence of portal hypertension. In China CSH is seen in patients who have chronic hepatitis and cirrhosis. Upper gastrointestinal hemorrhage was not only secondary to

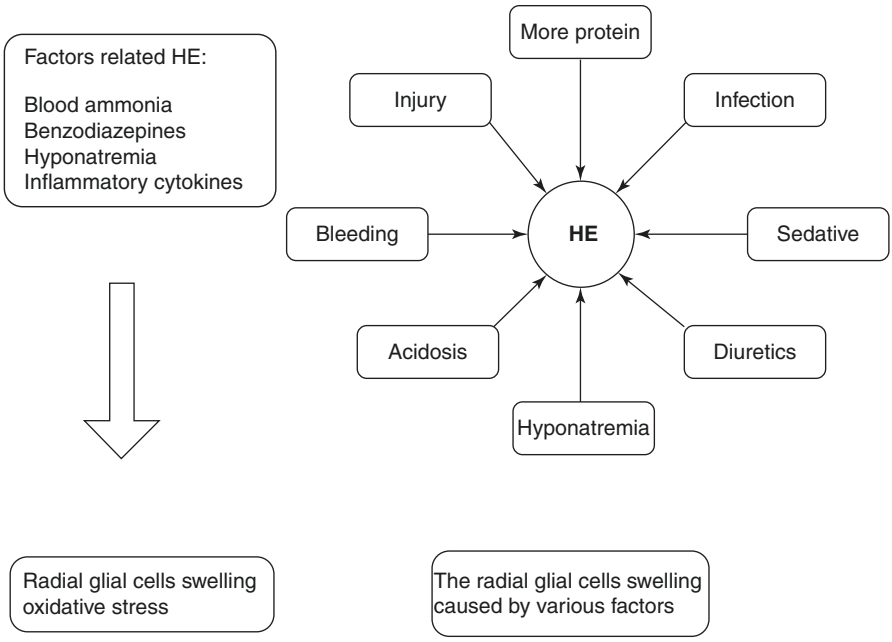

Fig. 6.2 The inducing factors of HE. Redraw from Häussinger D, Schliess F. Pathogenetic mechanisms of hepatic encephalopathy. Gut, 2008, 57(8):1156–1165 [17]

the presence of esophageal and gastric varices, but also by disorders in blood coagulation as a consequence of poor hepatic synthesis of clotting factors and infection. GI hemorrhage is a medical emergency which can lead not only to hypovolemic shock but also can precipitate hepatic encephalopathy (Fig. 6.2). HE can be seen in acute and chronic liver disease and in the setting of CSH is often a lethal event.

6.1.5.3 Acute Kidney Injury

AKI refers to a rapid deterioration in renal function as reflected by an increased serum creatinine to 26.4 mmol/L, or a rise of 50% from baseline (increased to 1.5 times). It is associated with a decrease in urine output (oliguria) to <0.5 mL/kg/h for more than 6 h. Traditionally, AKI has been divided into three categories as following, (1) pre-renal azotemia caused by inadequate renal perfusion without renal tubular and glomerular lesions; (2) renal tubular epithelial cell necrosis (ischemia or poisoning), glomerulonephritis, or interstitial nephritis leading to failure of renal parenchyma; (3) post-renal failure, hydro-nephrosis caused by urinary tract obstruction. Liver cirrhosis patients are likely to develop any type of AKI. Portal hypertension associated with cirrhosis causes splanchnic and systemic vasodilation, and a number of factors contribute to AKI including the release of nitric oxide (NO) which can cause vasodilatation and a decrease in systemic vascular resistance (SVR). Other systems that are invoked in patients with AKI include the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system, and the

non-permeable vasopressin release neuroendocrine system. As a consequence of insufficient blood volume water and sodium retention is increased leading to increased intravascular volume and cardiac output and ascites formation. Patients with established cirrhosis develop vasodilation, hypotension and tachycardia resulting in renal vasoconstriction and a subsequent decrease in renal blood flow. These changes in hemodynamics lead to the development of AKI which is a serious complication of severe liver disease, with high mortality. Patients with CRS also have a high incidence of HRS secondary to disturbances in hemodynamics. Clinically, it may be difficult to differentiate AKI from HRS.

6.1.6 Investigation of Long-Term Prognosis

Factors that affect the prognosis of patients with CSH are both complex and diverse. To accurately assess the severity and prognosis of CSH, it is important to carry out a thorough clinical examination as well as perform detailed laboratory tests. Ultimately however, the long term prognosis of patients with CSH is related to the development and severity of complications including GI bleeding, renal failure, hepatic encephalopathy and life threatening infections. Hepatic encephalopathy is a common clinical manifestation of CSH and often is a direct cause of death. Hepatorenal syndrome is also a serious complication and when it occurs in the late stage of CSH the prognosis is poor. Upper gastrointestinal bleeding is often a terminal event which aggravates pre-existing renal disease and induces HE. To improve survival, patients who develop CSH should be managed in specialized liver units where appropriate tests can be performed and medical treatment given to stabilize the patient. Once patients develop life threatening complications, only liver transplantation has been shown to be the only effective therapy to improve survival.

6.2 Prevention of Acute Exacerbation of Chronic Hepatitis B

Ming Wang, Dao-Feng Yang

Hepatitis B remains a serious worldwide infectious disease which is especially important in Asian countries. Although great progress has been made in understanding the pathogenesis and treatment of hepatitis B, there is still a lack of specific and effective treatment and thus its mortality remains high. Many patients chronically infected with HBV (CHB) will develop acute exacerbations (AECHB) which may be life threatening. Therefore, prevention of AECHB is critical to improve long term outcomes of patients with CHB. In addition, due to the complex natural history of HBV infection and the inability to predict the occurrence of AECHB, prevention of AECHB should include management and health care for patients in an inactive phase and effective interventions for patients in an active phase. Meanwhile, the complications should be prevented as far as possible to reduce the mortality rate in patients with severe hepatitis.

6.2.1 The Management and Health Care for Chronic Asymptomatic HBV Carrier Are the Basis for Prevention of AECHB

The natural history of HBV infection in infants is generally divided into four phases: the immune tolerance phase, the immune clearance phase, the low replicative phase (inactive phase) and the reactivation phase. Newborns with HBV infection account for only about 5%, since viral clearance may occur spontaneously. The majority of infected infants have a prolonged immune tolerance phase and then enter into an immune clearance phase. However, teenagers and adults with HBV infection will directly enter into the immune clearance phase without the immune tolerance phase. Most will spontaneously clear the virus (90–95%), and only a minority will (5–10%) develop HBeAg-positive CHB. Therefore we can prevent AECHB from these four phases, including preventing the transition of the immune tolerance phase into the immune clearance phase or active phase, which can lead to CHB and subsequently AECHB.

6.2.1.1 Management and Health Care for Patients in the Immune Tolerance Phase and the Inactive Phase

Patients with CHB and in the immune tolerant phase and the inactive phase usually have few if any clinical manifestations and thus do not seek medical help. However, some patients may progress into the immune clearance phase, resulting in the development of CHB and even severe hepatitis. Although liver inflammation and fibrosis of patients in the immune tolerant phase are often absent, many of these patients may have mild inflammation and fibrosis because of high levels of HBV DNA replication activity. In 2007, a 5-year follow-up study from Hong Kong University evaluated disease progression in 57 immune tolerant CHB patients. By the end of follow-up, 16% of patients had developed elevated serum ALT. Liver biopsies were performed in 48 patients who remained in the immune tolerant phase during 5 years of follow-up; 85% of the patients had no evidence of liver disease whereas 6.3% of the patients had disease progression [18]. Therefore, the results of this important study show that we should pay attention to patients in the immune tolerant phase.

Management of Patients

1. Regular examination: The following recommendations have been made for management of patients with CHB who are in the immune tolerant phase of disease: (1) Young (<30 years) patients with persistently normal ALT levels should be tested for ALT level every 3–6 months. (2) In patients with increased ALT levels, the test frequency of ALT and HBV DNA levels should be more frequent. HBeAg should be tested every 6–12 months. (3) No treatment is recommended for patients with mildly increased ALT levels except for patients who develop progressive fibrosis or cirrhosis, or patients with normal ALT levels who have histologic evidence of mild inflammation or fibrosis. (4) It is recommended that protocol liver biopsies be performed yearly in young patients who are in the immune tolerance phase. (5) Treatment should be initiated in patients more than

35 years of age if histological evidence of disease is verified, whereas in those patients who have no histological evidence of disease, ALT levels should be monitored every 3–6 months [19].

2. Diet and life habits: Although there is no special dietary restriction for patients who are immune tolerant. The study of Freedman et al. showed that the red meats such as pork and beef may be associated with an increased incidence of chronic hepatitis and may also lead to increased progression of chronic liver disease, whereas white meats such as fish and chicken may be associated with reduced incidence of chronic hepatitis. The mechanism for this observation is not known at this time. However, based on these findings, it is recommended that HBV-DNA infected patients should avoid excessive intake of red meats [20]. There were different reports on the relationship between smoking and hepatitis B. Sherman et al. reported that smoking is a major contributing cofactor that increases the risk of hepatocellular carcinoma development in CHB patients, and thus the CHB patients are advised to quit smoking [21]. Mota et al. reported that the use of alcohol especially in elderly patients with CHB is associated with more severe liver disease. Patients with alcohol intake <20 g/day had decreased inflammation and fibrosis on liver biopsy compared with the patients with alcohol intake >20 g/day. It is now appreciated that habitual drinking in patients with CHB infection leads to progression of disease including increased inflammation and fibrosis [22], Lin et al. reported that heavy alcohol consumption significantly increased the risk of HCC in HBV-related cirrhotic patients. The 10-year cumulative (52.8% vs. 39.8% vs. 25.6%, $P < 0.001$) and annual incidence (9.9%, 4.1%, and 2.1%) of HCC were significantly higher in cirrhotic patients with HBV infection who consumed excessive alcohol compared to patients with CHB who did not consume alcohol [23]. Thus, it is strongly recommended that patients with HBV infection quit drinking alcohol. In addition, HBV infected patients should do their best to ensure adequate rest, and attempt to reduce stress levels.
3. Providing further education for both the general population and patients about HBV infection would ensure that patients are not discriminated against and receive timely and effective access to care to improve long term physical and psychological outcomes.

6.2.1.2 Treatments in the Immune Clearance and Reactivation Phases of HBV Infection

When the patients are in the immune clearance phase, they have moderate or severe hepatic inflammation and necrosis and fibrosis can progress rapidly. Some patients may develop cirrhosis and liver failure as a consequence of immune activation. A sudden change in viral load may stimulate a strong immune response leading to immune clearance of infected hepatocytes, resulting in severe hepatitis. Most patients in the immune clearance and reactivation phases have mild or moderate liver function abnormalities and no obvious clinical symptoms of hepatitis and thus do not seek medical treatment. Others often ignore symptoms and signs of progressive liver disease, which leads to further disease progression including development

of liver failure or cirrhosis. Thus it is important that patients and physicians are educated, so that they can intercede in a timely fashion to prevent development of life threatening complications in chronically infected patients.

6.2.2 Active and Effective Treatment of Patients with CHB Are the Keys to Prevent AECHB

Epidemiological studies show that approximately 1% of patients with CHB develop severe hepatitis and the mortality rate of patients with severe hepatitis can be as high as 50–70%. Therefore, preventing disease progression in patients with CHB is the key to preventing AECHB. To improve outcomes of patients with CHB the following steps should be considered:

6.2.2.1 Close Monitoring of CHB Patients

All patients who have CHB should be closely monitored for any signs or symptoms of disease progression. Common symptoms associated with disease progression include fatigue, loss of appetite, nausea and vomiting, abdominal distension, oliguria or anuria and gradual mental deterioration. Signs of liver dysfunction include development of jaundice, bruising or bleeding, oliguria or anuria, hepatic encephalopathy and bacterial infection such as spontaneous bacterial peritonitis (SBP) or ascites. Patients should be monitored for disturbances biochemical or coagulation function, including measurement of levels of ALT, AST, total and direct bilirubin, INR and renal function (electrolytes and serum creatinine) [24].

6.2.2.2 Diet and Rest

For patients with CHB, rest can lead to improvement in hepatic microcirculation and hepatocyte regeneration which contributes to patient recovery. Studies have shown that hepatic blood flow is reduced by 40% when patients are in a prone (upright) position compared to when they are in a supine (recumbent) position. Hepatic blood flow can be further reduced by 80–85% when the person stands upright and carry out activities. The consequence of this alteration in blood flow is hepatic ischemia which further compromises the hepatic function of patients with CHB infection. As a consequence, the catabolism of glycogen and protein in the liver is increased and lactic acid production is increased, all of which contribute to further hepatic deterioration. Therefore, it is recommended that during the active phase of disease, patients with CHB get plenty of rest and resume normal activity only after recovery of liver function.

It has been suggested that patients with CHB take a low-fat, low-sugar and high-protein diet.

1. The daily energy requirement for a patient with CHB is 2000–2500 kcal. The food taken should be easy to digest, and the protein diet should be reasonably matched to increase the utilization rate of proteins, and ensure full absorption and utilization of amino acids. The protein requirement for adult is 0. 8–1.2 g/(kg day);

for children, pregnant women, nursing mothers and patients with malnutrition or chronic wasting disease, protein requirement may be increased to 1.5–2.0 g/(kg day). The protein intake should be reduced in patients with elevated blood ammonia or evidence of hepatic encephalopathy [25].
2. Intake of vitamins and trace elements: Foods should be rich in vitamins A, B and C. Studies have shown that vitamin D is beneficial in patients with cirrhosis and reduced levels of vitamin D are associated with severe hepatitis [26, 27].
3. As patients with HBV infection have digestive disease disorders, it has been suggested that frequent small meals are recommended, and overeating should be avoided.
4. Food prohibition: Patients should be strongly encouraged to avoid alcohol as it is known to directly damage the liver, and especially in patients with CHB. Chinese traditional medicine believes that hot and spicy foods should be avoided in patients with liver disease, and the intake of spicy food should be reduced.

6.2.2.3 Care and Psychological Guidance

Good care and positive psychological guidance may have an important beneficial role in improving patient recovery. The following measures should be carried out in the care and psychological intervention of the patients [28, 29]:

1. Regular follow-up can improve patient compliance through a variety of communication methods allowing the patient to fully understand, learn and master important health issues in CHB. This will help, patients understand the importance of proposed clinical treatment regimens to improve their health.
2. Psychological guidance: Counseling should be used to encourage patients to help reduce the psychological pressures associated with CHB. It is extremely important to engage friends and family members of the patients to enlist their help in support of these patients.
3. Patient health education: It is critical to educate patients with CHB about the importance of diet, rest, adherence to medication and follow visits with health physicians to improve their long term outcomes. Patients should be strongly educated to the importance of taking medications and in particular not to reduce the dosage or stop medications or to blindly seek unproven treatment without consulting with their physician.

6.2.2.4 Other Factors Influencing the Course of CHB

It is now known that there are many factors which may negatively influence the course of CHB. Zhao Zhengang et al. conducted a retrospective analysis to identify factors which cause acute-on-chronic liver failure in 289 patients who had CHB. The results suggested that: the HBV replication and mutations of HBV are the main causes of the development of acute-on-chronic liver failure (50.52%), followed by bacterial of fungal infection (24.57%), gastrointestinal bleeding (4.50%), drugs (4.15%), diarrhea (3.46%), use of alcohol (2.42%) and HEV superinfection (2.42%) respectively [30]. Other studies have reported that other causes of chronic hepatitis include fatigue, concurrent hyperthyroidism, pregnancy and abortion. Measures should be taken to reduce these conditions in order to prevent progression of HBV disease.

Prevention and Treatment of Secondary Infection or Superinfection

1. Prevention of bacterial and fungal infections: Due to decreased immune function, CHB patients have an increased susceptibility to a variety of bacterial and fungal infections which contribute to progression of HBV disease including development of liver failure. Specific measures to prevent bacterial and fungal infections must be adopted including patient education and avoidance of unnecessary surgical procedures. Aseptic techniques should be strictly adhered to when invasive operations must be performed. The use, dosage, course and administration route for antibiotics treatment should be controlled. As it is known that use of antibiotics is associated with an increase in fungal infections, patients on antibiotics should be screened for development of fungal infections, and once diagnosed, the treatment should be initiated promptly [31].
2. Prevention of other hepatotropic virus superinfection

   The patients with HBV can be superinfected by HAV, HCV, HDV, HEV, CMV and EBV. HBV/HEV is a common occurrence in patients with HBV disease and can lead to the development of AECHB. In addition, when HBV/HCV superinfection occurs, HCV can promote the development of chronic HBV. Patients with HBV who then become infected with HCV have a higher incidence of severe hepatitis.

   Patients with chronic HBV infection who have no immunity to HAV should be vaccinated against HAV. As epidemiologic studies have shown that human HEV infection in some regions comes mainly from infected pigs, individuals who raise pigs and work in slaughterhouses are at high risk of developing and transmitting HEV infection. Furthermore, it has been reported that pig liver can spread HEV to humans [32]. Therefore, attention should be paid to personal hygiene to prevent spread of HEV. Additional studies have reported that seafood and shellfish may be a reservoir of HEV and ingestion of raw shellfish may lead to HEV infection [30].

   Prevention of HBV/HCV superinfection: The main transmission routes of the HCV infection include: blood transfusion, sharing of syringes with infected persons, tattoos and rarely through sexual transmission. Prevention of HCV infection includes screening of blood, organ and tissue donors for HCV as well education of the population at risk of HCV transmission through sharing needles (drug addicts), at tattoo parlors and through body piercing. The development of effective anti-viral therapy should reduce and even eliminate the burden of HCV disease worldwide [33].

Avoidance of Hepatotoxic Drugs

The vast majority of drugs are metabolized in the liver and it is now known that patients with chronic liver disease have reduced or altered metabolic activity that leads to increased drug toxicity. Many drugs can cause increased liver damage and this is enhanced in patients with pre-existing liver disease. For example, use of antibacterial agents such as isoniazid and rifampin, antineoplastic agents, alkylating agents, pyretic analgesic and anti-inflammatory drugs and statin lipid-lowering drugs can lead to the development of AECHB in patients with CHB. The specific details of these reactions are discussed in detail in Chap. 6 of this book.

Treatment of Other Concomitant Diseases

Diabetes

Diabetes, especially poorly controlled diabetes, is closely related to the incidence and severity of AECHB [34]. Studies have reported that the diabetes is an independent risk factor for the development and exacerbation of advanced liver disease. Thus, close monitoring of blood sugar levels and antiviral treatment should be initiated in patients with CHB and diabetes to reduce the incidence of liver cirrhosis, liver cancer and liver failure and ultimately patient mortality.

1. It is important to treat the underlying liver disease in patients with hepatitis B-related diabetes. CHB patients should undergo timely and standardized antiviral treatment. During treatment of CHB patients, it is recommended to avoid hyperglycemia and maintain euglycemia which leads to an increase in response to anti-viral therapy.
2. Among the hypoglycemic drugs, the thiazolidinediones should be avoided in patients with active liver disease or in patients with liver transaminase levels greater than 2.5 times the upper limit of the normal range. As sulfonylureas can cause liver dysfunction and produce cholestasis, α-glycosidase enzyme inhibitors should be used with caution in patients with known liver dysfunction, and the use of biguanides is contraindicated in patients with liver dysfunction.

Hyperthyroidism

The association of hyperthyroidism with CHB usually leads to more severe clinical symptoms, jaundice and manifestations of hyper estrogenemia including presence of liver palms or spider angioma are commonly observed. Both hyperthyroidism and HBV infection can cause liver damage alone. Drugs for the treatment of hyperthyroidism including some traditional Chinese medicine can also easily lead to liver damage. Therefore, the presence of CHB combined with hyperthyroidism is more likely to result in AECHB, and management may be more difficult.

1. It is not recommended to use interferon therapy in CHB patients who have autoimmune disease including hyperthyroidism as it is well documented that the use of interferon may aggravate pre-existing autoimmune disease.
2. I^{131} treatment is first line therapy for treatment of hyperthyroidism, as surgical treatment is not recommended in patients with severe liver disease. The use of antithyroid drugs (ATD) is often associated with liver damage, although in most instances, the damage is mild and reversible especially upon cessation of the drug. The liver damage caused by methimazole (MMI) is associated mostly with drug dosage, while the liver damage caused by propylthiouracil (PTU) is not associated with the drug dosage. The liver damage can occur at any time after initiation of the drug, but is most commonly seen within 3 months after initiation, although it can occur as late as 1 year after starting these drugs. It can occur at any age, but is more commonly seen in women. The etiology is still not clear. A cohort study of hyperthyroidism patients receiving MMI/CBM or PTU showed that MMI vs PTU had a higher hepatitis incidence rate (3.17/1000 vs 1.19/1000

person-years) but a lower incidence of acute liver failure (0.32/1000 vs. 0.68/1000 person-years).
3. Most patients who develop liver disease secondary to use of antithyroid drugs are asymptomatic and have only mild disturbances in liver function. It is usually not necessary to stop taking these drugs, and antithyroid treatment can usually be continued at the same dosage. However, once liver abnormalities are found, it is recommended to closely monitor liver function. If the liver is significantly damaged, the drugs should be immediately stopped although interestingly it is has been shown that cessation of the drug does not necessarily lead to resolution of damage and some patients can go on to develop liver failure.

Tuberculosis

The use of antituberculosis drugs is commonly associated with hepatotoxicity. Some patients have only mild elevations in transaminases and are asymptomatic. In these patients, continued use of antituberculosis drugs can cause more severe liver damage and even acute liver failure.

1. The incidence of antituberculosis drug-induced liver damage varies from 2% to 28%, and factors that have been shown to affect incidence include ethnicity, geographical environment and diagnostic criteria. The incidence of isoniazid-induced liver damage is 0.5–4.0% with an average of 1.0% [35].
2. Liver function should be monitored closely at the early stage of anti-tuberculosis treatment, and anti-tuberculosis treatment should be carried out with caution especially in elderly patients with severe CHB. Once antituberculosis drug liver damage is diagnosed, it should be treated immediately. However, whether one should stop using antituberculosis drugs is decided according to the severity of liver damage. The American Thoracic Society recommends that if the serum ALT level >5 × ULN, or ALT >3 × ULN combined with symptoms such as significant jaundice and/or fatigue, loss of appetite, nausea, vomiting, jaundice, abdominal distension, right upper abdominal discomfort and hepatomegaly, the medication should be immediately stopped. After ALT is reduced to less than 2 × ULN, rifampicin and (or) ethambutol can be re initiated if ALT does not increase again 3–7 days later, the isoniazid can then be added. If ALT increases again or the symptoms of hepatitis reappear, isoniazid should be stopped [36].
3. For some patients with severe tuberculosis, stopping antituberculosis drugs also has the risk of causing death, the regimen without liver toxicity can be temporarily used as a transition, until the liver function returns to normal, and then the combined anti-TB treatment can be re-initiated. Marra et al. found that the incidence of liver damage in patients receiving the treatment regimen containing levofloxacin is lower than that in the patients receiving conventional anti TB regimens. The long-term application of fluoroquinolones has a good safety profile, especially the fourth-generation fluoroquinolones such as moxifloxacin which is known to have more effective anti-tuberculosis activity, and can be used as alternate drug therapy [37].

4. Prior to the start of anti-tuberculosis treatment, patients should be advised to stop drinking alcohol. It is important that liver function tests are monitored in patients who receive these medications as these agents can either directly cause liver damage or exacerbate pre-existing disease. Once abnormalities in liver function are found, the withdrawal of the drugs is usually associated with resolution of liver damage.

Pregnancy

Pregnancy can aggravate the clinical course of a CHB patient, and even cause liver failure. The asymptomatic HBsAg carrier during pregnancy is prone to develop active disease which could threaten the lives of both mother and fetus.

1. Female CHB patients of childbearing age who have therapeutic indication should be treated with IFN or nucleotide analogs and they should use reliable contraceptive measures during treatment [29].
2. If a patient gets pregnant while on oral antiviral therapy, with lamivudine or other class B drugs for pregnancy (telbivudine or tenofovir vinegar), the treatments can be continued. However, the doctor must fully inform patients about the risks of use of these medications to the fetus and weigh the pros and cons as well as have patients sign an informed consent [29].
3. When HBV virus infection occurs in patients during pregnancy, the decision to initiate anti-viral treatment is determined based on the severity of the liver disease. As lamivudine, telbivudine or tenofovir have been associated with teratogenesis, prior to initiation of therapy, doctors should fully inform patients about the risks of their use and weigh the pros and cons as well as have the patient sign an informed consent. Frequent monitoring of maternal and child health should be initiated in patients with CHB. In general, it is not necessary to abort the pregnancy in patients with mild symptoms, especially those who are already in mid-pregnancy. Vaginal delivery is the preferred mode of delivery for patients with CHB. If the liver disease worsens during pregnancy and threatens the life of the mother, termination of the pregnancy should be considered [29].

6.2.2.5 Anti-viral Therapy

Patients with CHB who satisfy the indications for treatment should receive antiviral treatment to inhibit the replication of HBV DNA which is known to reduce liver inflammation and fibrosis, delay the occurrence of decompensated liver cirrhosis and the development of hepatocellular carcinoma (HCC) and prolong the lives of patients. Specific anti-viral regimens and precautions will be discussed in detail in another chapter of this book. During anti-virus treatment, one should be cognizant about the occurrence of HBV activation and mutation, patient compliance and drug resistance and adjust therapy accordingly to prevent severe hepatitis. In addition, it is known that the use of interferon can produce liver damage and even precipitate severe life threatening hepatitis and thus it is critical that laboratory tests and clinical statuses of patients are monitored closely during treatment.

6.2.2.6 Comprehensive Treatment of CHB

The treatment of CHB consists of the use of anti-viral medications as well as medications that reduce inflammation and fibrosis and immune mediated injury.

Anti-inflammatory, Antioxidant and Liver Protecting Treatment

In addition to direct anti-viral treatment, use of agents that have anti-inflammatory and antioxidant activity also play important roles in the treatment of patients with CHB. Use of these agents can reduce inflammation and liver cell necrosis and protect the liver through multiple mechanisms such as stabilizing the hepatocyte membrane, enhancing the metabolism of hepatocyte enzymes, by promoting the repair and regeneration of hepatocytes there are a rich variety of these drugs in China.

1. Glycyrrhizin preparation: A large number of studies have shown that the use of glycyrrhizin can inhibit liver inflammation, as determined by reductions in serum aminotransferase levels and improvement in liver histology [38].
2. Liver cell membrane protective agents: Polyene phosphatidylcholine has been shown to have hepatic protection activity and reduce hepatic fibrosis. Silymarin can reduce levels of aminotransferase [39]. Bicyclol can eliminate free radicals and protect liver cell membranes and mitochondria, reduce inflammatory damage of the liver and prevent liver fibrosis. It can also enhance protein synthesis in liver and promote hepatic cell regeneration.
3. Other drugs enhancing liver cell metabolism: Detoxification and liver-protecting medicines as well as Chinese patent medicines are now being evaluated for potential benefit. The use of agents including glucuronolactone, tiopronin, bifendate and Wuling pill to reduce glutathione are now being studied. When selecting the use of adjuvant drugs for the treatment of liver disease, the use of agents which may cause liver damage or precipitate liver failure should be avoided.

Immunomodulation

The use of agents with immunomodulatory activity is expected to become an important means of treatment of CHB in the future. However at present there is a lack of specific immune therapies for hepatitis B which have been shown to have a positive affect on the course of disease. Drugs including thymosin-α1, thymopentin, lentinan and transfer factor [40] have been used in combination with antiviral drugs to improve the antiviral efficacy, although their benefits remain to be proven.

Anti-fibrosis Treatment

The use of agents that reduce hepatic fibrosis would be an important adjuvant treatment for CHB, especially for patients with established liver cirrhosis. Use of such agents potentially could prevent the incidence of HCC in patients with CHB. There is a wide range of oral anti-fibrotic agents in China including Anluohuaxian pills, Fuzhenghuayu capsule, Fufangbiejiarangan tablets, Biejiajian pills, Qianggan capsules, rhubarb Zhechong pills, compound 861, Handanbituo granules, kushenin capsule, Huoluoshugan capsules and Xiaochaihu that could be studied in prospective trials for potential efficacy [41]. Interferon-γ which has both anti-viral and

immunomodulatory activities has been approved for the treatment of liver fibrosis. Until proper studies are performed, the use of agents which might be of benefit to reduce fibrosis should be restricted to centers that have expertise in the treatment of CHB as it has been reported that many of these agents can induce liver failure.

6.2.3 Preventing the Occurrence of the Complications is the Most Important Remedy for the Patients Who Have Developed Severe Hepatitis

Patients with CHB and AECHB are prone to all kinds of complications, including the development of hepatic encephalopathy (HE), hepatorenal syndrome (HRS), infection and gastrointestinal (GI) bleeding. The occurrence of these complications is significantly associated with a poor prognosis. Thus these patients are best managed in specialized liver centers that can offer experimental innovative therapies and transplantation which has proven to be an effective treatment for patients with CHB.

6.2.3.1 Prevention of HE

The prevention of HE includes the following measures:

1. Patients should be educated about the causes of HE and in particular to avoid drugs, foods or other agents that can result in development of HE. Known causes of HE include gastrointestinal bleeding, electrolyte imbalance, use of sedatives and diuretics, large volume paracentesis and infection [42–44].
2. Maintain good bowel function and avoid constipation: Development of constipation is associated with HE. This is due to an increase in blood ammonia levels secondary to reabsorption of nitrogenous material in the colon. The use of agents like lactulose have proven to be effective in prevention and treatment of HE by acidification reducing NH_3 to NH_4 and preventing absorption of NH_3. Lactulose should be given to increase bowel movements to 2–3 per day [43].
3. Diet control: Patients should take an adequate diet to maintain good nutritional status. However, protein intake should be restricted in HE patients to with less than 20 g/day. Intake of vegetable protein as opposed to protein from red meats is thought to have less potential for development of HE. Supplementation of vitamins and trace elements is recommended in patients on protein restricted diets. All patients should avoid alcohol [43].
4. Although there is currently a lack of evidence on effect of the use of antibiotics, most people believe that early use of non-absorbable antibiotics is associated with the decrease of the occurrence of HE.

6.2.3.2 Prevention of HRS

The incidence of renal dysfunction is very high in patients with CHB. A serious complication is the development of HRS. The development of HRS is associated with high patient mortality. Therefore, prevention and early detection of HRS is important. Bacterial infections, excessive use of diuretics, large volume

paracentesis and upper gastrointestinal bleeding all can precipitate renal failure and HRS in patients with severe hepatitis. Once severe renal dysfunction develops, patients should be admitted to hospital preferably in a specialized liver unit. The diagnosis of type 1 or type 2 HRS can be confirmed by measurement of serum creatinine, renal volume and renal function and serum electrolytes. Treatment consists of careful monitoring of fluid balance, correction of electrolyte disturbances and renal replacement therapy including infusion of albumin and drugs (midodrine and octreotide) to correct abnormalities of volume status and improve blood flow to the kidney.

1. Prevention of bacterial infection: Up to 30% of patients with ascites due to liver cirrhosis develop spontaneous bacterial peritonitis which can progress to HRS [45]. The prophylactic use of antibiotics in combination with albumin treatment can reduce the incidence rate of bacterial peritonitis to 10% [46]. Furthermore, it's recommended the use of antibiotics in combination with albumin infusion in patients with spontaneous bacterial peritonitis (SBP) to reduce the incidence rate of SBP and HRS and increase the survival rate of patients [47–49].
2. Avoid excessive use of diuretics and large volume paracentesis: Maintenance treatment for patients with established liver cirrhosis includes sodium restricted diet and the use of diuretics. However, caution must be exercised using this treatment approach in patients with decompensated cirrhosis as manifested by presence of ascites and hyponatremia to prevent further renal dysfunction. AASLD guideline recommended that low volume therapeutic paracentesis can be performed in patients with refractory ascites. However if the quantity of ascites extracted at any one time is greater than 4–5 L, albumin infusion (6–8 g/L of ascites extracted) should be performed to prevent disturbances in renal function [47]. Other measures to be taken include maintenance of fluid and electrolytes and acid-base balance and avoidance of the use of nephrotoxic drugs.

6.2.3.3 Prevention of Infection

The development of bacterial or fungal infection is one of the leading causes of mortality in patients with CHB. Risk factors for the development of infections include the need for repeated paracentesis, reduced immunity associated with established cirrhosis, the prophylactic use of broad-spectrum antibiotics, need for hospital admission and increasing age.

1. Measures should be undertaken to correct hypoalbuminemia. Agents that could augment host immunity would be extremely beneficial and recently it has been suggested that thymosin can reduce the incidence rate of infection in patients with severe hepatitis and improve the survival rate of the patient [50].
2. Surgery in patients with chronic liver disease should be avoided unless lifesaving.
3. The antibiotics should be used cautiously to prevent drug resistance. With the exception of prophylaxis to prevent SBP, use of antibiotics prophylactically has not proven to be of benefit in patients with CHB infection.

6.2.3.4 Prevention of GI Bleeding

Patients with severe hepatitis have disturbances of blood coagulation as manifested by an increase in prothrombin and partial thromboplastin times (PT, PTT and INR) as well as a reduction in platelet counts secondary to presence of portal hypertension. The development of portal hypertension results in the development of portal hypertensive gastropathy or esophageal and gastric varices which can result in an increased risk of bleeding and the patients are prone to GI bleeding. Once the patients have GI bleeding, this may tend to further increase liver damage in patients with severe hepatitis, and lead to complications such as ascites, HE and HRS.

1. Eliminate risk of GI bleeding: Use of non-steroid antiinflammatory drugs which are known to cause GI bleeding should be avoided. In patients with abnormal coagulation indices (increased INR), surgical procedures should only be performed with use of plasma and platelet function.
2. The appropriate transfusion of blood plasma and supplement of blood coagulation factors are critical to prevent bleeding. Patients with severe thrombopenia (<50,000 platelets/mL) are transfused with platelets to reduce the risk of bleeding. The patients with severe hepatitis should be treated with vitamin K routinely.
3. Severe upper GI bleeding is a common and serious side effect in patients with CHB. It is recommended that proton pump inhibitors should be used prophylactically in CHB patients to prevent upper GI bleeding. The use of octreotide has been shown to reduce or stop active bleeding in CHB patients with portal hypertension.

6.3 New Research Directions in Acute-on-Chronic Liver Failure (ACLF)

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6.3.1 Background

Acute-on-chronic liver failure (ACLF) is a recently described syndrome which is characterized by sudden acute clinical deterioration, accompanied by multi-organ failure and a high mortality in patients with established liver cirrhosis [51–53]. ACLF may develop at any time during the course of chronic liver disease. The epidemiology, diagnostic criteria, clinical course, and prognosis of ACLF have recently been described in a large multicenter European prospective observational investigation, the CANONIC study [51]. The study included 1383 patients consecutively admitted to 29 European university hospitals for the treatment of acute decompensation of cirrhosis. Approximately 31% of patients admitted to the hospital with cirrhosis had ACLF at admission (20%) or went on to develop ACLF during the course of hospitalization (11%).

ACLF typically is known to occur in younger patients and often secondary to alcohol related disease or in non-treated hepatitis B virus (HBV) patients. In Asia, ACLF is a known complication of patients with chronic hepatitis B (CHB) [51]. In China, it is estimated that approximately 70% of liver failure is caused by HBV infections, and, therefore, the prevalence of HBV-ACLF will likely increase in the coming decades. There is evidence suggesting that severe acute exacerbation of CHB either over the natural course of the disease or following intensive chemotherapy or immunosuppressive therapy is an inevitable stage in the development of ACLF. In recent years, emphasis has been placed on identifying the cause of ACLF. It has been suggested that systemic inflammation with release of pro-inflammatory cytokines secondary to sudden infection, deterioration of renal function and GI bleeding in patients with liver cirrhosis is the cause of ACLF. Thus, effective control of these factors maybe crucially important to slow down or reverse the progression of acute exacerbation of CHB to ACLF.

Clinical manifestations of HBV-ACLF are nonspecific and may include fatigue, jaundice, nausea, and vomiting. Biochemical parameters include elevated levels of alanine aminotransferase (ALT), total bilirubin, and INR. However, similar disturbances in liver biochemistry are also observed during acute exacerbation of CHB without ACLF and therefore relying on these conventional parameters may not allow for the early diagnosis of ACLF and rapid initiation of appropriate management.

Presently, there are no effective medical therapies for patients who develop ACLF [52]. However, patients with this condition should be admitted to a specialized liver unit with expertise in their management. The use of extracorporeal liver support systems to remove inflammatory mediators and reduce inflammation including the molecular adsorbent recirculating system (MARS) and Prometheus have in general been unsuccessful although their use may temporarily prolong the life of patients until liver transplantation is performed [52]. Although alcohol related disease is the major cause of ACLF worldwide, in Asia, ACLF is more commonly associated with HBV infection, and thus we will confine discussion of research opportunities for ACLF in the setting of HBV infection.

There are over 2 billion people worldwide who have been infected by HBV and over 350 million people are chronic carriers [54]. Persistently infected patients are at risk of developing ACLF and hepatocellular carcinoma [51, 52, 55]. HBV persists in infected hepatocytes, because covalently closed circular DNA (cccDNA), the template for the transcription of viral RNAs, is stable in nondividing cells [56]. Although antiviral therapy inhibits HBV DNA synthesis in infected hepatocytes, it does not destroy nuclear cccDNA and thus new strategies must be developed to eliminate cccDNA [57]. Furthermore, it is well known that failure to develop an appropriate protective innate and adaptive T and B cell immune response leads to persistence of HBV and development of CHB. New strategies to eliminate HBV cccDNA through enhancement of the immune response or novel anti-viral therapy as well as development of new approaches to inhibit systemic inflammation is essential to improve outcomes for patients with ACLF secondary to HBV infection (Fig. 6.3).

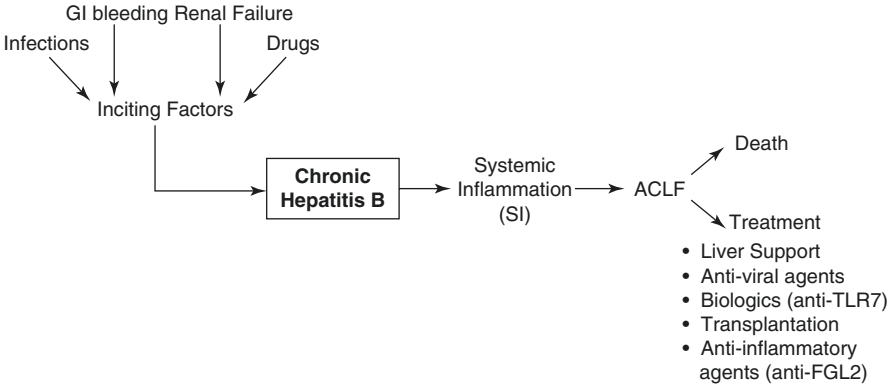

Fig. 6.3 Precipitating causes and Treatment of Acute on Chronic Liver Failure (ACLF) in Patients with HBV Infection

6.3.2 Future Research Directions

6.3.2.1 New Approaches to Clinical Care

Patients in the early stages of ACLF should be admitted to specialized liver units with the capacity to perform liver transplantation to improve survival [58]. This will allow both for optimal clinical care but also for collection of patient samples and data which will advance approaches to ACLF. Presently use of extracorporeal liver support systems including albumin dialysis by the molecular absorbent recirculating system (MARS) has had little impact on improving long term survival [59–61]. However, the use of MARS results in improvement in patient hemodynamics and stage of hepatic encephalopathy. Thus, additional well controlled studies using present or new liver systems are warranted. Other approaches which could be studied include the use of granulocyte colony stimulating factor to treat infections as well as the potential of use of stem cells to restore immune integrity and enhance liver regeneration [61, 62].

6.3.2.2 Development of New Cell and Animal Model Systems

One of the impediments to development of understanding the pathogenesis and developing effective therapies for HBV has been the lack of suitable animal models. More recently, using molecular techniques cell lines and animal model systems have been developed which will allow greater understanding of the molecular biology of HBV, the immune response and the development of novel therapies (Table 6.5).

The availability of HBV transfected cell hepatoma/ hepatocyte cell lines (HepG2, Huh7 and HC04); the HepG2.2.15 cell line that has a stably integrated HBV genome and the infectable hNTCP-HepG2 cells should allow for studies to examine the effects of new anti-viral agents on HBV replication prior to performing preclinical studies in small animal models and human clinical studies. In addition, the availability of large well defined patient cohorts who have cleared HBV or developed

Table 6.5 Humanized mouse models

Mouse model	Mechanism	Use	References
Hu-PBL-SCID	Engraftment of CD3+ human T cells	Studies of human Immune function	[63]
HU-SRC-SCID	Engraftment of human hematopoietic stem cells	Immune Studies	[64]
SCID-HU	Engraftment of fetal liver and thymus	Immune studies and liver pathogenesis	[65]
HU-Tg Mice	Transgenic expression of Human Genes	Human Gene Function	[66]
A2/NSG/NODCg-Prkdc^{scid}IR2rg$^{tm1wjl/s2J}$	Reconstitution of human immune system and human liver	HBV replication, immune pathogenesis and therapy	[67]
HBV-Tg Mice	HBV Plasmids	HBV replication and immune studies	[68]

Hu-PBL-SCID human-peripheral blood leucocytes-severe combined immunodeficiency disease, *Hu-SRC-SCID* human-scid repopulated cell-severe combined immunodeficient disease, *SCID-HU* severe combined immunodeficient disease-human, *HU-Tg Mice* human transgenic mice, *NSG* nod scid gamma, *NOD* non obese diabetic, *HBV-Tg* hepatitis B Virus transgenic mice

CHB or HBV-ACHD will allow for studies to define changes in the immune repertoire and response as well as gene expression studies and studies of viral replication. All of these well-defined cells will be useful for analysis of the effect of new therapeutic agents on viral replication before proceeding to more costly studies in humanized animal models of HBV, chimpanzee or clinical trials in human.

Studies of mouse models of acute and chronic hepatitis including Mouse Hepatitis Strain 3 (MHV-3) [69] and Lymphochoriomeningitis virus (LCMV) WE and CL 13 [70–72] have provided important insights into the pathogenesis of human hepatitis including HBV and HCV. The importance of pro-inflammatory mediators including FGL2-prothrombinase to massive hepatic necrosis during MHV infection as defined by Ning et al. and PD-1/PD-1L to T cell exhaustion and failure to develop multi-lineage CD4 and CD8 and B cell protective anti-viral responses leading to failure of clearance of LCMV has been extended to patients with CHB caused by HBV and HCV [72, 73]. Furthermore, the relevance of regulatory T cells (Treg) and production of immunosuppressive cytokines IL-10 and FGL2 in suppressing anti-viral immunity has now been shown in patients with chronic HBV and HCV disease [74].

The availability of humanized and transgenic HBV mouse models will now allow studies of HBV replication and immunopathology (Table 6.5). Standardized methodologies to generate humanized mice have now been recently developed and published [68, 75]. For example, the recent description of immune-deficient mice bearing a mutant IL-2 receptor gamma chain (IL2rγ) has greatly facilitated the engraftment of human hemato-lymphoid cells as well as other cells and tissues. Engrafted mice develop normal human immune systems with fully functional T and B cells. These mice can be engrafted with human hepatocytes which then can be infected with HBV allow for studies of viral kinetics and the effect of the immune

system on viral clearance and development of chronic disease. A humanized mouse model with both a human immune system and human liver cells by reconstituting the immune-deficient A2/NSG/NOD.Cg-*prkdc*^{scid}*IL2rg*^{tm1wjl}SzJ mice with human HLA-A2 HSC/Hep supported HBV infected cells has now been generated [66]. HBV infected and reconstituted mice had impaired immune responses, chronic liver inflammation and liver fibrosis. Thus, these mice potentially could serve for testing of new anti-viral agents and biologics for their impact on viral eradication and restoration of anti-viral immune response, which can then hopefully be translated to humans.

6.3.2.3 Targeting HBV

To date, eradication of HBV has not been achieved and anti-viral agents serve only as viral suppressors which must be taken life-long [76]. Thus, it is critical to identify new targets that can lead to viral eradication. The ultimate goal of new research efforts must now be directed to HBV eradication similar to what has been accomplished with HCV infection. One high priority is to silence or remove cccDNA. Another promising approach is to study the microRNA (miRNA) profiles that occur during viral replication, in patients that clear the virus and those that develop chronic disease. The use of real time PCR panel analysis can be utilized both in cell lines such as the HepG2 cells and in primary hepatocytes isolated from patients to identify target miRNAs that may inhibit HBV replication and may prove to be useful therapeutics. Ultimately, agents that are effective can then be studied for efficacy first in humanized or transgenic mouse models before being used in patients in clinical trials. A number of effective anti-viral agents including interferons and nucleos(t)ide analogues have led to control of HBV infection [77]. However, these therapies generally do not lead to eradication of virus and thus new strategies are now being explored to alleviate the burden of HBV disease worldwide (Table 6.6).

6.3.2.4 Approaches to Enhance Immunologic Clearance of HBV Infection

The availability of large cohorts of well-defined patients especially in Asian countries will now allow investigators to identify mechanisms of viral clearance as well development of chronic disease. Gene signatures can be obtained from patients enrolled in these studies and targets identified for development of both biomarkers of response as well as new bio-therapeutics. Furthermore, by expanding these studies to include patients from different parts of the world, the importance of genetic variation in outcome to treatment can be identified.

The introduction of mass cytometry by time of flight (CyTOF) is a new form of flow cytometry that utilizes high molecular weight isotopes as tags instead of fluorophores [86, 87]. This allows for simultaneous acquisition of up to 49 independent cellular parameters, which will allow for analysis of cellular heterogeneity. CyTOF has recently been reported to extensively map cell subsets which will now allow fine mapping of epitopes and immune responses through high throughput analysis of both PBMC and intrahepatic lymphocytes from patients with CHB. This will advance our further understanding of the role of host innate and adaptive immune

Table 6.6 New approaches to eradicate HBV

Technology	Targeted use	Reports to date	References
CRISPR/Cas9	cccDNA (HBV)	Partial Success in HepG2 cells in vitro and zygotes in vivo	[78, 79]
miRNA	IFN mediated innate immunity	Decrease in HBV gene expression/replication	[80]
Myrcludex B	Inhibits HBV entry	Maximum 10% reduction in HBV replication	[81]
Anti PD-1	Restores T and B cell anti-viral immunity	Elimination of LCMV	[82, 83]
Anti IL-10/ IL-10R	Restores anti-viral immunity/clearance of LCMV	LCMV infection	[71]
Anti FGL2/ FcγRIIB	Restores anti-viral immunity/clearance of LCMV Prevents fulminant hepatitis by MHV	LCMV chronic infection MHV induced FHF	[72, 84]
TLR7 agonists	Increase innate and adaptive anti-viral immunity	Loss of HBsAg	[85]

LCMV lymphocytic choriomeningitis virus, *IL-10* interleukin 10, *CRISPR/Cas9* clustered regularly interspaced short palindromic repeats Caspase 9, *miRNA* micro Ribo nucleic acid, *IFN* interferon, *cccDNA* covalently closed circular DNA, *HBV* hepatitis B virus, *HBsAg* hepatitis B surface antigen, *HCV* hepatitis C virus, *FGL2* fibrinogen like protein 2, *FcγRIIB* Fc gamma receptor RIIB, *TLR7* toll like receptor, *PD-1* programmed cell death protein 1

responses both in clearance of HBV spontaneously or following institution of treatment and in the development of chronic hepatitis. Specific subsets of $CD4^+$ and $CD8^+$ HBV specific T cells can be identified through the course of disease as well as response to therapy and the role of Treg subsets in inhibiting effector responses ascertained. CyTOF can be combined with cytokine analysis to further assess the HBV specific T cell repertoire and in particular the magnitude and quality of the T cell response to a specific antigen. These studies will ultimately lead to identifying immune targets and development of more effective biologics, which can impact various aspects of the immune system.

A number of immune-modulators have been identified that have important roles in the pathogenesis of experimental acute fulminant hepatitis (MHV-3) [88, 89] and acute and chronic viral hepatitis caused by LCMV (LCMV WE and LCMV Cl 13) as well as human HBV and HCV hepatitis [70, 71, 74, 83, 90]. In patients with CHB, increased numbers of $FoxP3^+$ Treg have been reported [91]. Interference with IL-10 and its receptor IL-10R; PD-1L and its receptor PD-1; and FGL2 and its receptor FcγRII/IIIB have all lead to clearance of LCMV and inhibition of T cell exhaustion. Treatment with agents that inhibited these pathways led to restoration of normal T and B cell long term immunity to LCMV and clearance of the virus. Furthermore, in a model of fulminant viral hepatitis caused by murine hepatitis virus strain 3 (MHV-3) by Ning et al. and acute hepatitis caused by LCMV WE antibody to FGL2 led to improvement in liver histology and clearance of virus [69, 72]. If proven successful, interference of Treg and immune inflammatory mediators

might be of use in treating patients with ACLF. These agents could now been tested in a humanized animal model of HBV both to confirm their usefulness and to gain understanding of their effects on the immune response to HBV prior to undertaking clinical studies. Finally, preclinical studies have suggested that TLR7 agonists (GS9260, Gilead Sciences) lead to loss of HBsAg in the Woodchuck model of HBV and sustained viral suppression in Chimpanzees. These agents could be further studied in HBV cell lines as well as in the humanized model of HBV infection to confirm their efficacy.

6.3.2.5 New Anti-viral Therapies

Existing therapies for HBV only target the viral polymerase and generally are unable to eradicate HBV. New approaches and agents to therapy for HBV are being developed. These include trials of combination anti-viral therapy in previously untreated patients; use of new agents that potentially could disrupt cccDNA; microRNAs that could interfere with HBV replication and immune-modulators that could potentially restore effective T and B cell anti-viral immunity.

A number of new therapeutic agents including TLR7 agonists (Gilead Science) [92, 93], viral entry inhibitors such as Myrcludex B [81] and HBV core inhibitors such as NVR 3-778 (Novira Therapeutics), are in development and could be tested in humanized animal models for efficacy before entering clinical trials in CHB patients [94]. A recent report of a Phase 2A trial of Myrcludex B in patients with chronic HBV showed that a dose of 10 mg was well tolerated and there was a 10% reduction at week 12 in 75% of treated patients, which was maintained until week 24 of treatment. Exciting results of reduction/elimination of HBV in HepG2 cells using new technologies including CRISPR Cas9 and microRNA have recently been reported and thus may have great therapeutic potential [78, 79].

6.3.2.6 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

An exciting new approach to disrupt cccDNA is to develop clustered regulatory interspaced short palindromic repeats based RNA-guided nucleases (CRISP/Cas9) targeting the HBV sequence [95]. Potentially this approach would lead to viral eradication by disrupting cccDNA. One group has reported that cleavage and repair of HBV cccDNA by Cas9 in HepG2 cells leads to a single nucleotide insertion and deletion. Larger deletions were observed but at a lower frequency [78, 79]. HepG2/NTCP cells expressing the single guide RNA exhibited an eight to tenfold reduction in the number of HBcAg expressing cells and by PCR it was shown that approximately 90% of cells carried the mutation but as many as 8% of cells remained wild type. The results of these experiments suggest that a single insertion/deletion might be insufficient to completely inactivate cccDNA. The most common mutation observed in these studies was a single nucleotide deletion at position-4. A number of recent reports have shown the utility of using this approach in-vivo, but to date only partial success has be achieved and only in the zygote. Unfortunately, the ability to eliminate cccDNA in adult hepatocytes using CRISPR has not been achieved. Thus newer RNA guided targeted DNA recognition systems will need to be developed if this approach is to be clinically useful.

6.3.2.7 microRNAs

MicroRNAs (miRNA) are small RNA that bind to the untranslated region (UTR) of target RNA and either positively or negatively regulate their translation [80]. Recent studies have identified miRNA that regulate IFN-mediated antiviral innate immunity by directly targeting the viral RNA. Specifically, a number of recent studies have reported that microRNAs have been shown to affect HBV gene expression and inhibit HBV replication [96–98]. A recent study has reported that 24 highly differentiated miRNA were identified in HepG2.2.15 cells versus HepG2 cells. Following IFN alpha treatment, 23 differentially expressed miRNA were identified in HepG2 cells whereas only 5 miRNA were found in HepG2.2.15 cells. This suggests that HBV impairs miRNA expression and specifically impairs IFN inducible miRNA responses in HepG2 in vitro. Further studies are warranted to determine the miRNA profiles during HBV replication using real time PCR panel analysis. Furthermore, using this approach, dysregulated miRNAs can be selected for their ability to suppress HBV replication first in cells lines such as the HepG2.2.15 which contain integrated HBV prior to conducting studies in human cells from patients with CHB. Subsequently, in vivo studies could then be conducted if transfection with miRNA mimics could inhibit HBV DNA replication in humanized animal models.

6.3.3 Conclusion

ACLF as a complication of chronic HBV infection is an important life threatening condition with a high mortality. Presently there are no effective medical therapies for patients who develop ACLF and liver transplantation often is the only therapeutic option for patients. The availability of new molecular tools including CyTOF, genomic technologies and multiplex ELISAs will provide further insights into the immunopathology of ACLF. Furthermore, new techniques including CRISPR Cas9 and microRNA technology have the potential to eradicate HBV cccDNA leading to elimination of HBV in patients with chronic HBV and reducing the incidence of ACLF. The availability of new biologics to prevent T cell exhaustion may lead to restoration of effective anti-viral T and B cell responses. The availability of humanized and transgenic mouse models of HBV will now allow testing of new biologics both to reduce inflammation and to promote eradication of HBV prior to testing in humans. These areas of research and approaches have the potential to markedly reduce deaths from acute on chronic liver failure (ACLF).

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