Main Complications of AECHB and Severe Hepatitis B (Liver Failure)

2

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Abstract

This chapter describes the clinical features, and diagnosis of complications in AECHB including secondary bacterial infections, coagulation disorder, water electrolyte disorder, hepatorenal syndrome, hepatic encephalopathy, hepatopulmonary syndrome and endotoxemia

1. Patients with severe hepatitis have impaired immunity and are therefore vulnerable to all kinds of infections. After infection, these patients may experience shock, DIC and multiple organ failure, all of which seriously affect their prognosis and are major causes of death. Concurrent infections consist primarily of infections of the lungs, intestines, biliary tract, and urinary tract, as well as spontaneous bacterial peritonitis and sepsis.

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2. Severe hepatitis may reduce the synthesis of coagulation factors and enhance their dysfunction and increase anticoagulants and platelet abnormalities, leading to coagulopathy. Infection, hepatorenal syndrome and complications can further aggravate coagulopathy, resulting in DIC and seriously affecting patient prognosis.
3. Hepatorenal syndrome, which is characterized by renal failure, hemodynamic changes in arterial circulation and abnormalities in the endogenous vascular system, is a common clinical complication of end-stage liver disease, and one of the important indicators for the prognosis of patients with severe hepatitis.
4. Water electrolyte disorder (water retention, hyponatremia, hypokalemia, hyperkalaemia) and acid-base imbalance are common in patients with severe hepatitis. These internal environment disorders can lead to exacerbation and complication of the illness.
5. Hepatic encephalopathy is a neurological and psychiatric anomaly syndrome based on metabolic disorder, and an important prognostic indicator for patients with severe hepatitis.
6. The hepatopulmonary syndrome is an important vascular complication in lungs due to systemic hypoxemia in patients with cirrhosis and portal hypertension. The majority of patients with HPS are asymptomatic. Long-term oxygen therapy remains the most frequently recommended therapy for symptoms in patients with severe hypoxemia.
7. Endotoxemia, an important complication of severe hepatitis, is not only a second hit to the liver, but also leads to other complications including SIRS and MODS.

2.1 Section 1: Severe Hepatitis Complicated with Infection

Jian-Xin Song and Lin Zhu

2.1.1 Introduction on Severe Hepatitis Complicated with Infection

Patients with acute or chronic severe hepatitis are extremely prone to be infected due to weakened immunity, leading to shock, DIC and multiple organ failure in a very short time, which is a main cause of death. Patients with severe hepatitis can be infected with bacterium or fungi, a few of them even get multiple sites infection or continuous infection by different pathogens. Pathogens in the infection of severe hepatitis include bacterium, fungi, mycobacterium tuberculosis, protozoon, virus etc. Clinical manifestations include pulmonary infection, intestinal infection, biliary tract infection, urinary infection, spontaneous bacterial peritonitis (SBP) and sepsis. Complicated infections directly influence the prognosis of severe hepatitis, also become one of the primary death cause.

2.1.1.1 Incidence of Severe Hepatitis Complicated with Infection

Patients with severe hepatitis are at high risk of infection. By collecting data from 18 centers of North American Consortium for Study of End-stage Liver Disease

(NACSELD) for survival analysis of prospectively enrolled cirrhosis patients hospitalized with infections, Bajaj found that there is 15.8% of nosocomial infection in all 507 patients [1].

Domestic data showed significant difference of infection incidence in different times. It is related to raising diagnosis and treatment level. According to the data from Shanghai Public Health Clinical Center, in 372 patients who were treated as severe hepatitis from January 2007 to December 2009, there were 68 patients obtained nosocomial infection with a rate of 18.3% [2].

The incidences of infection complicated with acute liver failure, acute on chronic liver failure or chronic liver failure are similar. Patients with old age or long hospitalization are more easily to get infection. There was rare report about infection in children patients with severe hepatitis. In 2011, Godbole et al. from UK reported that 25% in 145 children patients with severe hepatitis were complicated with infection, and mostly are sepsis, respiratory tract infection and urinary infection. Most of the infections occurred within 2 weeks of admission, while patients with infection had longer hospitalization [3].

2.1.1.2 Mechanism of Severe Hepatitis Complicated with Infection

Reduced Ability to Clear Bacterium

Reduced Innate Immunity

The innate immune system is the first line of defense against premier environmental challenges and injury. In liver, it is a complex system that includes NK cells, NKT cells, KCs, neutrophils, eosinophils and complement components. The innate immune response acts much more rapidly compared with adaptive immunity.

Monocytes and macrophages: The liver plays an important role in defense and immune function. The main cellular components of the innate immune system within the liver are the Kupffer cells. Kupffer cells represent 80–90% of the tissue macrophages in the human body [4]. In normal condition, Kupffer cells in liver help to clear the macromolecular substance such as pathogen, endotoxin, heteroantigen and immune complex to defense infection. In severe hepatitis, due to massive hepatocytes necrosis, the number and function of monocytes/macrophages are impaired, thus the activity of fibronectin, which is opsonin of macrophages, is decreased. Therefore, bacterium, endotoxin and other poison from gut directly access into circulation. Subrat Kumar Acharya from India reported that the plasma fibronectin (FN) level in severe hepatitis patients was significantly lower than that in healthy controls (85.6 μg/mL ± 75.8 μg/mL, vs. 295.5 μg/mL ± 88.5 μg/mL). The FN level was remarkably correlated with incidence of infection and mortality [5].

Complement: Liver is the organ where complements are mainly produced, such as C2, C3, C4, C5. The complements help to expand phagocytosis of phagocytes by chemotaxis, opsonization or adhesion, as well as help antibody to kill or solute some gram negative bacilli [6]. Report from Wyke showed that the defect of complement closely correlated with impaired opsonization [7]. Defect of C3 or C5 can result to weakened movement of polymorphonuclear leucocyte. In severe hepatitis,

the ability of liver to produce complement has been weakened due to massive injury of parenchymal hepatic cells, which leads to decreased activity of complement to 40% of normal condition. Meanwhile, serum opsonization to E. coli or saccharomycetes are also diminished. Besides, high ammonia level in severe hepatitis also restrains complement activity to impact germicidal effect.

The most direct and also the most important result for decreased complement production and reduced opsonization is the susceptibility to infection.

It was also reported that complement and the alternative pathway play an crucial role in LPS/D-GalN-induced fulminant hepatic failure [8].

Neutrophils: A majority of patients with severe liver disease have altered function of neutrophil granulocytes [9]. The most common manifestation include abnormal ultrastructure or function of neutrophil granulocytes, as cytoplasm degeneration, organelle reduction, mitochondrial swelling, pyknotic nuclei, etc. Decrease filtration and phagocytosis of reticuloendothelial system, as well as impaired chemotaxis of blood cells, making immunity weakened, lead to invasion of bacterium. Therefore, patients with severe hepatitis are vulnerable to be infected with bacterium or fungi due to decreased phagocytosis and germicidal effect of neutrophil granulocytes, and impaired adhesion of macrophages and white cells [10].

Data from Liu H demonstrated pretreatment neutrophil-lymphocyte ratio was associated with the prognosis of patients with HBV-ACLF, and elevated NLR predicted poor outcome within 8 weeks [11].

Natural killer and natural killer T cells: Natural Killer (NK) and Natural Killer T cells (NKT) are important components of the innate immune response. Natural killer cells have potent cytolytic activities that are exerted through the death receptor and perforin/granzyme pathways. Activated NKT cells have both perforin-dependent and Fas-Ligand dependent cytotoxic function that are triggered upon TCR recognition of an antigen [12].

NK cells and NKT cells play an important role in many experimental models of liver injury, such as viral hepatitis, alcoholic liver disease, and autoimmune liver disease [13]. However, their role in ACLF has not yet been clearly elucidated, It was reported the median percentage of NK cells in the lymphocytes of patients with acute and fulminant liver failure were significantly lower compared to healthy controls. Meanwhile, patients with acute and fulminant liver failure had significantly high and comparable NKT cells compared to control group [14].

Reduced Specific Immunity

The important pathophysiological role of innate immune dysfunction in patients with acute-on-chronic liver failure (ACLF) has been investigated in recent years. However, dysregulation of adaptive immunity remains poorly elucidated [15].

Patients with severe hepatitis has varying degrees of impaired cellular immunity, manifested as decreased CD4+ cell number and declined CD4+/CD8+ ratio, which is pathogenesis of opportunistic infection.

It was reported that there exists a reduction in CD4(+) T lymphocytes in HBV-ACLF patients. These CD4(+) T cells predominantly are CD4(+) Tconv, and the development of suppressive CD4(+) Tregs greatly surpass Tconv, which constitutes

important characteristics of adaptive immune dysfunction of HBV-ACLF [15]. A report from China showed total amount of lymphocytes, CD4(+) T cells, CD8(+) T cells and NK cells in circulation were lower in the HBV-ACLF patients compared to the CHB patients [16].

HBV-specific CD8(+)T-cell responses are considered to be of great importance in viral control and immune-mediated liver damage [17]. However, CD8(+) T cell has seldom been studied in ACLF. Ye [18] reported decreased activated CD8(+) T cells may be related to poor outcomes in patients with SH.

The frequency of circulating Th17 cells increased with disease progression from CHB to ACLF patients compared to healthy control. Th17 cells were also found largely accumulated in the livers of CHB patients. The increases in circulating and intrahepatic Th17 cells were positively correlated with plasma viral load, serum alanine aminotransferase levels and histological activity index. In addition, the serum concentration of Th17-associated cytokines was also augmented in both CHB and ACLF patients [19].

Impact of Invasive Diagnosis and Treatment

In process of diagnosis and treatment for severe hepatitis, repeatedly abdominal paracentesis, retention catheterization, venous cannula, hemofiltration, or trachea cannula are usually necessary. Unthoroughly sterilization or nonstandard sterile operation will lead to pathogen invading to develop an infection. In addition, artificial liver treatment is also an important cause of infection. It was reported that the incidence of fungal infection in severe hepatitis correlated with the number of artificial liver treatment.

Application of Broad Spectrum Antibiotics

Application of broad spectrum antibiotics is also a major cause of infection in severe hepatitis. Antibiotics inhibit or kill sensitive normal bacterium as well as pathogens, especially normal bacterium colonized in natural orifice, leading to flora disproportionality. This time, nonpathogen could cause infection, or mass produced pathogen become dominant colony to develop infection. It was proved that dosage, exposure time, or varieties of antibiotics used in patients was closely correlated with severity of dysbacteriosis and incidence of SBP [20]. Nosocomial origin of infection, long-term of norfloxacin prophylaxis, history of recent infection by multiresistant bacteria and recent use of β-lactams were independent inducements associated with the development of multiresistant infections.

Bacterial Translocation

Bacterial translocation is defined as the migration of viable microorganisms or bacterial products (i.e., bacterial LPS, peptidoglycan, and lipopeptides) from theintestinal lumen to the mesenteric lymph nodes and other extraintestinal sites [21]. There are multiple mechanisms which are involved in defective gut functions and altered microbiota in patients with cirrhosis or liver failure. These include small intestine bacterial overgrowth (SIBO), increased intestinal permeability, and impaired antimicrobial defense. Additionally, decreased bile acids, due to decreased

synthesis and defective enterohepatic circulation, contribute to altered gut microbiota [22].

Small intestinal bacterial overgrowth (SIBO) was defined as $\geq 10^5$ total colony-forming units per milliliter of proximal jejunal aspirations, which presents in 59% of cirrhotic patients and is associated with systemic endotoxemia [23]. In the condition of hepatic failure, due to impaired immunity, bacterium overgrowth and translocation happened, which increased incidence of infection [24]. In vivo study from Wang showed phagocytosis of monocytes were prominently and immediately diminished after resection of liver tissue up to 90%, accompanied by decreased oxygen ingestion [24]. Mass propagation of E. coli in the lower part of the small intestine and bacterium translocation were all happened 2 h after resection. In rats with ascites in severe liver disease induced by carbon tetrachloride, the bacteria easily transferred from the intestine to extra-intestine, including local lymph nodes and blood circulation. These evidences proved that liver failure prompted the proliferation of intestinal bacterial overgrowth and translocation, which may be the main reason for the endogenous infections in patients with liver failure.

Another reason for bacterial translocation is that patients with cirrhosis or liver failure display increased intestinal permeability. In patients with severe hepatitis, gastrointestinal mucosa membrane has impaired ability for regeneration and repair, leading to dysfunction of intestinal barrier and declined anti-infection ability. Patients with severe hepatitis have weakened regeneration and repair capacity of gastrointestinal mucosa, as well as decreased gastrointestinal barrier function and resistance to infection. The intestinal mucosa often manifested as congestion, edema or erosion. Especially when intestine get infection, bacteria can invade to abdominal cavity directly or through slight mucosal defect [25]. Besides, ascites often present in severe hepatitis patients, which provide a good medium for bacteria to multiply.

Patients with severe hepatitis often have ascites combined with portal hypertension. Due to lobular structural damage, abnormal construct of liver sinusoids or blood vessels, bacteria directly access into the systemic circulation without filtration and phagocytosis by liver, leading to formation of bacteremia. Meanwhile, bacteria in blood circulation may access into the abdominal cavity and cause abdominal infections. Hence, patients with severe hepatitis are not only prone to get endogenous infection but also prone to have intestinal endotoxemia.

Decreased Ability to Resist Pathogens

Patients with severe hepatitis have decreased bile secretion, changes of bile composition, so that infection are prone to occur in epithelium of bile duct and gallbladder. In patients with severe hepatitis, intestinal edema and cellulitis are obvious, some may develop acute serositis. For patients with portal hypertensive gastrointestinal disease, regeneration and repair capacity of gastrointestinal mucosa are decreased, accordingly, the natural immune barrier function is reduced. Once esophageal and gastric venous bleeding occur, gastrointestinal ischemia aggravate, which result in decreased resistance to infection.

2.1.1.3 Risk Factors for Infection in Severe Hepatitis

Invasive procedures, complications, prophylactic use of broad-spectrum antibiotics, underlying diseases, long hospital stay, and old age are risk factors for infection in patients with severe hepatitis. Elderly patients with other underlying diseases, decreased immune function, more severe primary disease, have a high risk of infection and may predispose to severe infections. In addition, albumin level is closely related to ascites production and SBP occurrence. Irrational uses of immunosuppressive agents such as corticosteroids suppress immune function, lead to flora, and promote the formation of drug-resistant strains and pathogenic bacteria. Mortality of severe hepatitis is closely related to infection, which directly affects the prognosis of severe hepatitis. To reduce the incidence of infection in patients with severe hepatitis, we should aim at a variety of risk factors, improve patients' immunity, rectify hypoproteinemia, treat the primary disease, prevent complications, strengthen disinfection and isolation, operate with strict aseptic technique and strict indications for invasive procedures, and use antibiotics rationally.

2.1.1.4 Clinical Significance and Outcome of Concurrent Infection in Severe Hepatitis

Once patients with severe hepatitis are infected, the consequences are often serious, as infection easily diffuse, which is difficult to control. After infection, the bacteria produce more toxins, which aggravate liver disease and cause a series of adverse effects, finally lead to severe complications (hepatic encephalopathy, hepatorenal syndrome, and etc.), which is the major cause for death in patients with severe hepatitis.

Bacterial endotoxin plays a major role in development and prognosis of severe hepatitis when infection happened [26]. Endotoxemia may directly or indirectly worsen liver injury. Intrahepatic cholestasis positively correlated with endotoxin levels. Endotoxin can trigger hepatorenal syndrome. For kidney, endotoxin is a potent renal vasoconstrictor substance, which can make a strong contraction of the renal artery and renal blood flow reduction. In addition, endotoxin can cause kidney capillary thrombosis and acute renal tubular necrosis, or even renal cortex necrosis. Endotoxin can activate coagulation factor VII and factor VI, making the intrinsic coagulation system startup, as well as directly damage hepatocytes which release tissue thromboplastin to start the extrinsic coagulation system. Meanwhile, endotoxin can also damage the vascular endothelial cells to cause bleeding, especially upper gastrointestinal bleeding. In the event of gastrointestinal bleeding, infections are more prone to happen or primary infection aggravates.

In recent years, due to extensive use of broad-spectrum antibiotics, the mortality of severe hepatitis has been significantly reduced. However, infection is still an important cause of death. Nosocomial infection control directly affects the prognosis of patients with severe hepatitis, which is an important part for the treatment of severe hepatitis. Once the etiology and other evidence of infection appear, patients should be treated with antibiotics. Effective prevention and treatment for nosocomial infections are positive to promote recovery and reduce mortality.

2.1.1.5 Clinical Types and Pathogens of Severe Hepatitis Complicated with Infection

Local data showed that the most common infection in severe hepatitis are SBP or pulmonary infection, followed by urinary tract infection, biliary tract infection, gastrointestinal infection, and bloodstream infection [27].

The most common pathogen is bacterium, while fungi is also common. Clinical studies have shown that, Gram-positive bacterial infection, mainly are Streptococcus pneumoniae, tended to increase. Gram-negative bacteria play an important role in infections of abdomen, urinary tract and lower respiratory tract, mainly are Escherichia coli, Klebsiella and other gram-negative bacteria [27].

The common fungal infections are Candida albicans, Aspergillus, Sporothrix, histoplasmosis, coccidioidomycosis monocytogenes and etc. [28]. Cryptococcus and mucormycosis are rare.

2.1.2 Bacterial infection

2.1.2.1 Pathogenic Characteristics

Pathogens isolated from patient varied. Pulmonary infections are usually caused by Staphylococcus aureus, Staphylococcus epidermidis, or Enterococcus faecalis, sometimes by vice Haemophilus influenzae, Pseudomonas aeruginosa, or Streptococcus. Infection of intestinal, biliary tract or peritonitis are most common caused by intestinal bacteria. Escherichia coli may account for about 50% of these infections, while Enterococcus, Klebsiella pneumoniae, Streptococcus and anaerobic bacteria infections are also reported. The pathogens for sepsis often come from lung, intestine, biliary tract or abdomen with a broader spectrum.

2.1.2.2 Clinical Manifestation

Spontaneous Bacterial Peritonitis (SBP)

Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid (AF) in the absence of a contiguous source of infection and/or an intra-abdominal inflammatory focus [29]. Sources of infections include biliary, intestinal and urinary tract, where are more likely to have inflammation or obstruction. Patients with severe hepatitis are extremely prone to acquire SBP. In 1960s, mortality of SBP was as high as 90%. At present, due to early diagnosis of the disease and rational use of antibiotics, the mortality dropped to 15–30%. Recurrence rate in survivors from SBP within 1 year is 40–70%.

Pathogen

Pathogens of SBP are always from intestine, accounts 70% of infection. E. coli is the most common, followed by Klebsiella pneumoniae, Streptococcus pneumoniae and other Streptococcus and Enterococcus. Staphylococcus is rare, accounting for about 2–4% of patients, which was seen in umbilical necrosis erosion. Anaerobic and microaerophilic bacterium are rare.

Pathogenesis

Bacterial translocation (BT) is the key mechanism in the pathogenesis of SBP, which is because of the concurrent failure of defensive mechanisms in liver failure [30]. Investigators have demonstrated pronounced impairment of gastrointestinal tract motility in cirrhosis [31]. The disturbance of gut flora microecology was associated with changes in the (ultra)structure of the gastrointestinal tract [32]. Meanwhile, reduced cellular and humoral immunity make it easier for the free flow of microorganisms and endotoxins to the mesenteric lymph nodes [33].

Besides, it was reported that hypoalbuminemia was related to SBP. A prospective study from Runyon reported that the incidence of SBP in patients with ascites protein less than 1.0 g/L was 15%, while the number in patients with ascites protein more than 1.0 g/L was only 2%. After 3 years of follow-up, SBP incidence in patients with ascites protein higher than 1.0 g/L were negligible [34]. The level of ascites protein correlates with opsonin activity, which means ascites protein <1.0 g/L was an independent risk factor for SBP.

Clinical Manifestations

The clinical manifestations of SBP are diverse. Most patients have subtle and insidious onset, which usually manifested as abdominal pain and fever. Acute onset may burst chills, fever and abdominal pain. Generally the body temperature of patient is around 38 °C, sometimes as high as 40 °C. Fever type usually presents as remittent fever. Abdominal pain is always around the navel or lower abdomen, paroxysmal or persistent. Nausea and vomiting are obvious, sometimes with diarrhea. Ascites can occur in patients without ascites, or increased significantly. Patients may also have significant bloating, abdominal muscle tension, tenderness, rebound tenderness, diminished or disappeared bowel sounds, and so on, or even shock in severe cases.

According to clinical manifestations, SBP can be divided into five types:

1. Common type: Acute onset, protruding abdominal pain, followed by fever. Or irregular fever take place followed by abdominal pain, abdominal tenderness and rebound tenderness, mild to moderate abdominal tension and growing ascites. Increased WBC count and nuclear left shift. Routine examination of ascites showed acute inflammatory changes.
2. Shock: Septic shock break out in a few hours to one day after abdominal pain or fever. The clinical manifestations include low temperature, lip cyanosis, abdominal tenderness, and hardly relieved shock. WBC count increases, blood culture is positive.
3. Encephalopathy: Fever and abdominal pain are often obscure, the early emergence of trance and other neuropsychiatric symptoms rapidly develop into a coma. Careful examination of the abdomen in patients with light coma may still find in patients with pain expression. Jaundice is deep, liver damage is serious.
4. Refractory ascites: Ascites progressively increase, which is difficult to subside with invalid diuretic therapy. Abdominal distension is obvious, often

without pain. Carefully check the abdomen may still find slight peritoneal irritation.
5. Asymptomatic: Symptoms are mild, exhibited as slight bloating, occasional fever. Mild tenderness can be found by deep palpation.

In addition, a considerable part of the patients showed non-specific symptoms and signs, such as deepened coma, deepening jaundice, oliguria, azotemia or dramatically increased ascites. Diagnosis of SBP should be considered if following conditions exist: (1) fever, which can't be explained by other reasons or other parts of infection; (2) abdominal pain, abdominal tenderness or rebound tenderness, but not serious; (3) sudden increased ascites or poor diuretic effect manifested as refractory ascites; (4) sudden onset of septic shock; (5) rapid deterioration of general condition, or deteriorated liver and kidney function in a short term, deepening jaundice, or hepatic encephalopathy.

Laboratory Tests and Diagnosis

The diagnoses of SBP mainly rely on ascites puncture. EASL clinical practice guidelines recommend that a diagnostic paracentesis should be performed in all patients with new onset grade 2 or 3 ascites, and in all patients hospitalized for worsening of ascites or any complication of cirrhosis (Level A1) [34].

For patients with severe hepatitis, diagnostic paracentesis indications are:

1. in liver cirrhosis patients with ascites that paracentesis should be performed after admission to determine whether SBP exist.
2. if the following conditions happened during hospitalization, diagnostic paracentesis should be performed: (1) abdominal sighs suggest abdominal infections, such as abdominal pain, rebound tenderness and gastrointestinal symptoms (such as vomiting, diarrhea, intestinal paralysis); (2) systemic infection signs such as fever, leukocytosis, or septic shock; (3) no clear incentive for hepatic encephalopathy, or rapidly emerged renal dysfunction.
3. in patients with ascites and gastrointestinal bleeding, paracentesis should be performed before prophylactic antibiotics.

Once ascites was acquired, polymorphonuclear cells (polymorphonuclears, PMN) count and ascites culture should be performed. Diagnosis of SBP is made according to international guidelines [34, 35] in patients with liver cirrhosis if the ascites polymorphonuclear (PMN) cell count exceeds 250 cells/μL and other forms of peritonitis have been excluded.

An ascites fluid polymorphonuclear (PMN) leukocyte count $\geq 250/mm^3$ should be considered as SBP, if PMN $>500/mm^3$ can be confirmed as SBP. If there is bloody ascites (erythrocytes more than $10{,}000/mm^3$), PMN count are as 1/250 of red blood cells.

The leukocyte esterase reagent strips (LERS) test is based on the esterase activity of the leucocytes. Since 2000, 26 studies have examined the validity of using leukocyte esterase reagent strips (LERS) in SBP diagnosis. LERS appeared to have low

sensitivity for SBP. On the other hand, a high negative predictive value (>95% in the majority of the studies)supported the use of LERS as a preliminary screening tool for SBP diagnosis [31].

SBP VARIANTS

1. **Bacterascites**

There is bacteria colonization in ascites but no inflammation. The diagnosis is based on positive ascites culture, ascites PMN countless than 250/mm^3, without evidence of systemic or local infection. Bacterascites have two outcomes: a short-term or transient bacterial ascites (mostly asymptomatic), or the development of SBP (mostly with symptoms). Once the diagnosis was established, paracentesis examination should be conducted again after 2–3 days. Take appropriate action under the circumstances. If the second sample has a PMNL count >250/mm^3, treat as for SBP. If the PMNL count is<250/mm^3 and a second set of cultures is positive, treat as for SBP. If the PMNL count is <250/mm^3 and the second set of cultures is negative, no further action is recommended [36]. If ascites culture is positive but ascites PMN <250 /mm^3 and there were signs of abdominal infection, it usually progress to SBP within a few days. These patients should be given appropriate antibiotic therapy.

2. **fulminant** Onset of Spontaneous Bacterial Peritonitis

Brolin first proposed the concept in 1982. The mechanism: in early stage of severe hepatitis, ascites has not been exist yet, however because of necrosis of hepatocytes, dysfunction of Kupffer cell, impairment of liver immune barrier, intestinal bacteria easily invasive into the systemic circulation through liver, causing spontaneous bacteremia, and then SBP.

Diagnostic criteria: (1) primary disease was severe hepatitis, no ascites was detected by strict examination and ultrasound. (2) clinical manifestation include fever, varying degrees of abdominal pain, diarrhea, diffuse abdominal tenderness and rebound tenderness, increased peripheral blood leukocyte, positive Rivalta's test, ascites fluid leukocyte count>500/mm^3, or PMN > 250/mm^3. (3) exclude abdominal organ perforation or primary foci.

3. **Culture** Negative Neutrocytic Ascites (CNNA)

It was used to describe the clinical situation when the ascitic PMNL count is >250/mm^3 but cultures fail to grow any bacteria. However, the term is now considered obsolete.

Pulmonary Infection

In severe hepatitis with secondary infection, pneumonia is most common. Patients with hepatic encephalopathy are susceptible to pulmonary infections as pneumonia since bed-ridden, impaired cough reflex and inadequate ventilation, especially

comatose patients with intubation and tracheotomy. Inpatients underwent thoracentesis, paracentesis and other invasive procedures, non-specific damage to immune barrier provide conditions for the bacterial invasion. Reported in patients with invasive procedures, lung infection risk increased significantly, which demonstrated blood infection is an important way for pulmonary infection. Furthermore, there was a significant increase of pulmonary infection in patients with intestinal infections or abdominal infection.

Pathogen: In recent years, pneumonia was classified as "community acquired pneumonia" (community acquired pneumonia, CAP) "and" nosocomial pneumonia (hospital acquired pneumonia, HAP). CAP is the Pneumonia acquired outside hospital [37]. Although CAP can be caused by a wide variety of micro-organisms, the pneumococcus, Mycoplasma pneumoniae and Chlamydophila pneumoniae, Staphylococcus aureus and certain Gram-negative rods are more usual pathogens encountered [38]. HAP is the Pneumonia that develops 48 h or more after hospital admission and that was not incubating at hospital admission. HAP infected with Gram-negative bacilli accounts for more than 60%, of which Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, other aeruginosa are common bacteria. The primacy is Pseudomonas aeruginosa, while gram-positive cocci accounted for about 20%, mainly are Staphylococcus aureus, coagulase-negative staphylococci, viridans and Streptococcus pneumoniae. Anaerobic is rare.

Clinical manifestations and diagnosis: Clinical manifestations are characterized as fever, cough, expectoration, dyspnea, and cyanosis.

Diagnostic criteria:

1. chest percussion of dullness, auscultation of rales, along with one of the following conditions: (1) purulent sputum or change of phlegm; (2) positive pathogens in sputum culture.
2. The chest X-ray and/or CT examination revealed new or progressive exudative lesions, and the emergence of one conditions above.

Urinary Tract Infections

Urinary tract infection is divided into upper urinary tract infection and lower urinary tract infection. Upper urinary tract infections are mainly pyelonephritis, which can be manifested as acute pyelonephritis and chronic pyelonephritis. Lower urinary tract infections include urethritis and cystitis. The most common pathogen of urinary tract infection is Escherichia coli, followed by Enterococcus faecalis, Klebsiella pneumoniae and Streptococcus agalactiae. Urinary tract infections often occur in patients with indwelling catheter, therefore, aseptic urethral catheterization and management and timely replacement of catheterization are effective to prevent such infections [39].

Clinical manifestations

1. acute pyelonephritis: (1) acute onset; (2) chills, chills; (3) fever; (4) malaise, headache, fatigue; (5) loss of appetite, nausea, vomiting; (6) urinary frequency,

urgency, dysuria; (7) low back pain, kidney area discomfort; the (8) tenderness of upper ureter point; (9) tenderness of rib waist point; (10) percussion pain in kidney area or bladder.

2. chronic pyelonephritis: (1) acute onset of acute pyelonephritis with the same, but usually much lighter, even without fever, malaise, headache and other systemic manifestations, urinary frequency, urgency, dysuria and other symptoms are not obvious; (2) edema; (3) hypertension.
3. urocystitis and urethritis: frequent urination, urgency, dysuria, urinary bladder pain. Urethral secretions.

Laboratory tests and diagnosis:

Diagnosis elements include: (1) tenderness in rib waist point, percussion pain in kidney area. (2) urine leukocytosis, pyuria. (3) urinary sediment smear find bacteria. (4) positive in urine culture. (5) urinary colony counts>10^5/mL; in patients with urinary frequency and other symptoms, colony counts>10^2/mL are meaningful; counts 10^3–10^4/mL also helpful in diagnosis; (6) one hour Urine WBC count>200,000. (7) blood test showed leukocytosis, neutrophil nucleus left. (8) increased ESR.

Biliary Tract Infection

Biliary tract infection is a common complication in severe hepatitis, which is often in company with cholelithiasis to reinforce each other. Hepatitis B virus can directly violate bile duct cells and cause cholecystitis. On this basis, cholelithiasis and secondary bacterial infections are easy to happen and become a major focus, which can cause other parts of infection in severe hepatitis patients. Furthermore, severe hepatitis patients often have reduced gastric acid secretion, so that E. coli in duodenum easily reproduce and cause ascending infection. Biliary tract infection is usually a mixed infection of aerobic and anaerobic infections. Enteric gram-negative bacteria include Escherichia coli, Klebsiella, Enterobacter, Proteus and Enterococcus. Anaerobic Bacteroides include Clostridium and Fusobacterium, in which Bacteroides is common, about 80–90%, particularly Bacteroides fragilis.

Common symptoms of biliary tract infection include chills, fever, nausea, vomiting, right hypochondrium pain and tenderness in gallbladder area. Clinical performance of concurrent biliary tract infection in severe hepatitis is not always very clear, often unconfirmed by pathogen test. Symptoms were epigastric or right upper quadrant pain, radiating to the right shoulder, hours after a heavy meal or a high fat meal. The patient may have severe colic, often accompanied by nausea and vomiting. Patients with chronic biliary tract infection have indigestion symptoms as heartburn, belching, acid reflux and bloating, or sometimes fever and right upper quadrant pain.

Intestinal Infection

Severe hepatitis patients have weakened intestinal resistance, especially for reduced immunoglobulin A secretions, which creates good invasion opportunities for bacterium. In addition, as mentioned above, intestinal flora are prone to happen in severe

hepatitis patients, which makes intestinal infection very common [24]. Few patients exhibit cellulitis like colitis, which can further result in peritonitis and septicemia, and finally lead to death. Pathogens in intestinal infections could be Shigella spp., Salmonella, Campylobacter jejuni, Clostridium difficile, and Salmonella typhi etc.

Clinical manifestations are nausea, vomiting, abdominal pain, diarrhea, watery stool or bloody mucopurulent stool. Some patients may have fever and tenesmus. Depending on the pathogenesis and clinical manifestations, intestinal infections are classified as enterotoxigenic bacterial enteritis and invasive bacterial enteritis. Pathogenesis of enterotoxigenic bacterial enteritis is that bacteria adhere but not invade intestinal mucosa. Intestinal toxins are secreted in the process of bacteria growth and reproduction, which stimulate small intestinal epithelial cells secreting large amount of water and electrolytes. Overuptake of water and electrolyte and retention in intestine makes watery stool, which is called "secretory diarrhea." Secretory diarrhea is exhibited as frequent, large amount stool with no pus, usually without pain or tenesmus. It is often accompanied by vomiting, which is prone to bring out dehydration, electrolyte imbalance and acidosis, but less severe systemic toxic symptoms. Stool examinations show less erythrocytes or leukocytes. Invasive bacterial enteritis refers to pathogenic bacteria adhere and invade the intestinal mucosa and submucosa, causing significant inflammation. Different pathogens violate different parts of intestine, small intestine, or colon, and sometimes cause inflammation both of small intestine and colon. The basic clinical manifestations include obvious systemic sepsis, high fever, and even septic shock in severe patients. Stool can be mucus bloody or purulent, less amount and more frequency. Abdominal pain are often severe, paroxysmal colic. If the lesion invades the rectum and distal colon in particular, there may be tenesmus. Sigmoidoscopy examination showed diffuse inflammation and ulceration. If only the small intestine or upper part of colon are invaded, the stool is more moisture, and without tenesmus. Stool examination show large amount of leukocytes.

Although diagnosis of intestinal infection is not difficult, it should be careful to distinguish the site of infection, make sure of pathogens, and pay particular attention to water, electrolyte imbalance and acid-base imbalance. Therefore, in addition to routine examinations and stool culture, keeping abreast of the general condition is also important to avoid disturbance of the internal environment which aggravate liver damages.

1. **Sepsis**

In severe hepatitis, the more severe hepatic damage and immune dysfunction, the higher of incidence of sepsis. Bacterium most commonly enters through intestine into the portal vein and then into the systemic circulation, followed by skin, respiratory tract, urinary tract or other intrusion. Pathogens are often opportunistic bacteria, in which gram-negative bacteria are more than gram-positive bacteria, especially Escherichia coli.

Clinical manifestations of nosocomial infection concurrent with severe sepsis are not specific, easily masked by the primary disease and complications. In some

cases primary focus is not obvious, therefore the diagnosis mainly rely on blood culture. Clinical manifestations include: (1) unexplained sudden chills, fever, shock, increased peripheral blood leukocytes or neutrophils; (2) deepening jaundice, an increase in ascites, or appearance of hepatic coma, hepatorenal syndrome in a short term. When complications appear in severe hepatitis patients, sepsis should be alert. The mortality of sepsis in severe hepatitis is high. Nosocomial infection not only aggravates liver damages, but also induces hepatic coma, hepatorenal syndrome, upper gastrointestinal bleeding and other serious complications, leading to multiple organ failure. Nancy Rolando reported mortality of septicemia in patients with liver failure was as high as 59%, in which 98% with septic shock [40].

2.1.3 Fungal Infection

2.1.3.1 Pathogenic Characteristics

Fungal infections can be classified into endogenous and exogenous infection. According to the source of the fungus, the former belongs to opportunistic pathogens, while the latter is in the environment, being infected through various routes of exposure. Fungal infections in severe hepatitis are invasive fungal infection in most occasions, and the majority are nosocomial infection and endogenous pathogenic fungi infection [27]. Candida infection is most common, followed by Aspergillus, again neoformans and Histoplasma monocytogenes. Candida albicans is widely present in normal human digestive tract. Other fungi such as Cryptococcus neoformans, Aspergillus are widely found in nature, which can colonize in body surface, or non-enclosed cavity. They also exist in hospital work environments, increasing the chance of nosocomial infection in hospitalized patients. Clinically, severe hepatitis with fungal infections are mostly superinfection.

2.1.3.2 General Situation and Influencing Factors

The rate of fungal infection in severe hepatitis is increasing in recent years. Nancy Rolando reported fungal infection was present in 16 of 50 acute severe hepatitis patients (15 Candida, 1 Aspergillus) and in seven cases was considered the major cause of death [41]. Domestic data reported that in a group of patients with liver failure, fungous infection was found in 143 cases in which the rate of nosocomial infections was 86.71%. In 155 separated fungous strains, 90 strains (58.06%) were Candida albicans, 17 strains (10.97%) were Aspergillus fumigatus and 25 (16.13%) strains were non-Candida albicans. The main sites of fungus infection were lungs (94 cases) and oral cavity (53 cases) [29].

For severe hepatitis complicated by fungal infection, the mechanism is complex. There are many influencing factors such as immune dysfunction and reduced defensing ability, besides, application and abuse of broad-spectrum antimicrobial drugs are also related [42]. Because of broad-spectrum antibiotics destroy the imbalance between bacteria and fungi in digestive system, which inhibit the growth some gram-negative bacteria that had an anti-fungal effect and some bacteria that are able to synthesize vitamin B family. Lack of vitamin B can lead to inhibition of

oxidation coenzyme, enabling weakened immunity, which is conducive to fungal growth.

Research and experience have demonstrated that repeatedly use of glucocorticoid is also an important factor to induce fungal infection in patients with severe hepatitis [28]. And therefore, the use of corticosteroids also need special care of. Currently the use of glucocorticoids in patients with severe hepatitis is still controversial. The majority do not advocate glucocorticoids, or consider a short-term use in the early stages of the disease and be removed as soon as possible. Otherwise it will cause a large increase of possibility of fungal infection.

Fungal infections are among the leading causes of death in patients with severe hepatitis. According to an analysis from Rolando, among 11 cases of severe hepatitis, seven cases of deaths directly related to fungal infections [41]. A recent domestic research analyzed outcomes of 115 patients with severe hepatitis, it was found that the mortality of patients with fungal infection was significantly higher than those without fungal infections (59.1% compared to 34.8%) [28].

2.1.3.3 Clinical Manifestation of Deep Fungal Infection in Severe Hepatitis

According to statistics, fungal infections often occur eight days after admission (0–24 days) or 5.5 days after broad-spectrum antibiotics usage (1–14 days). Symptoms of fungal infection are often covered by severe liver injury related symptoms, while clinical systemic manifestation such as fever is also difficult to identify with the bacterial infection. The site of infection often occurs in mouth, respiratory, digestive or urinary tract. Severe immunocompromised persons may appear disseminated infection.

Oral Fungal Infection

Oropharyngeal is the site that fungal infections mostly occur, and Candida albicans is the most common pathogen, followed by non-Candida albicans and Aspergillus infection. Bedridden patients with severe hepatitis can not properly maintain oral hygiene, which results in change of oral local environmental pH and blood flow. Candida albicans easily retains in the mouth, and multiplies, causing oral flora and opportunistic infections. Usually general symptoms are mild. Patients often have abnormal taste or loss of taste, followed by xerostomia, mucosal burning and other symptoms. Candida stomatitis may have pseudomembrane formation which is not easy to peel, accompanied by angular cheilitis, or sometimes manifests as mucosal congestion, erosion or clumps shrink of tongue papillae, thickening of coating on the tongue. There are clear lines between oral pseudomembranous damages. If remove the pseudomembrane it will leave a bright red base, sometimes thick film is like a layer of cheese. Take the film directly under microscopic examination shows hyphae and spores. Oral fungal infection is often a prelude of deep fungal infection, which should be on the alert. Simple oral Candida albicans infection is not always accompanied with fever. Oral fungal infection is easy to be found, therefore, if oral fungal infection exists with fever and increased leukocytes, it should be paid attention to merger of deep fungal infections such as the lungs or other organs [43].

Pulmonary Fungal Infection

Aspergillus is ubiquitous in nature which can be spread through air flow. The most common pathogen that causes invasive aspergillosis is Aspergillus fumigatus, while Aspergillus flavus, Aspergillus niger and Aspergillus soil are less common. Inhalated Aspergillus spores can reproduce in healthy or immunity weakened people. Respiratory tract is an infective route of invasive aspergillosis, accounted for 95%. Once tissue infection exists, blood vessels violation and bloodstream invasion are common. Invasive aspergillosis infection has three characteristics: tissue necrosis, hemorrhage, dissemination [44].

The mortality of invasive pulmonary aspergillosis accompanied by severe hepatitis is up to 90% [44]. It is lack of specific clinical manifestations. In most patients fever is the first arisen symptom, mostly with middle or low heat, sometimes with sudden high fever. Hemoptysis occurs with fever simultaneously or 1–8 days later, often accompanied with purulent tan sputum or bloody sputum, sometimes with a pinhead size of gray-green particles in it. Shortness of breath and chest tightness often exist in late stage of infection, as well as dyspnea, cyanosis, hypoxemia, and expectoration of a lot of bright red bloody sputum or blood clots. Pulmonary signs appear later, manifests as pulmonary wet or dry rales, occasional pleural friction sound. In some cases there are no pulmonary signs.

X-ray examination of invasive pulmonary aspergillosis showed patchy infiltrates and (or) nodular lesions. Typical nodules were like cotton, which can occur in unilateral or bilateral lobes. The lesion progresses rapidly to cause expanded infiltrates, and segmental or lobar consolidation. CT examination showed mass shadow, nodules, or exudative lesions. There are two typical imaging performance: (1) A characteristic finding on chest CT is the halo sign: a solid nodule surrounded by a halo of ground-glass attenuation [45]. (2) the formation of voids, which appear in late infection. Signs are hollow cavity lesions, the balloon “crescent” sign was seen around necrosis tissue [46, 47].

Digestive Fungal Infection

In order to prevent hepatic encephalopathy, patients can be given high doses of laxatives such as lactulose, which can reduce the intestinal pH value, creating an environment for growth of fungi, thereby increasing the chances of fungal infection of the intestine. In particular, some antibiotics combination increases the incidence of intestinal candidiasis. Patients usually have watery or jerry-like diarrhea, with foam or blood. Diarrhea is accompanied by bloating, sometimes with vomiting and fever, however, abdominal pain is not obvious. In Patients with severe hepatitis, due to decreased intestinal mucosal defense capability, Candida may invade the muscle and cause intestinal bleeding, or even perforation. In part of patients, it may even progress to fungal peritonitis, which is similar to clinical manifestations of bacterial peritonitis. In patients with oral candidiasis, if there is dysphagia or pain, especially retrosternal burning, it should be considered that esophagus is invaded. Incoordination motor of the upper and lower esophageal can be found by Esophageal barium enema. Gastroscopy helps to confirm the diagnosis.

Urinary Fungal Infection

Urinary fungal infection usually involves bladder and kidney, among which Candida infection is the most common. However, all pathogenic fungi (such as Cryptococcus neoformans, Aspergillus species, Mucor species, histoplasma, blastomycete, coccidioides) can spread to the urinary system as a systemic or part of disseminated fungal infection, which is more related to usage of broad-spectrum antibiotics and indwelling catheters. Clinical manifestations include fever and urinary tract irritation, while some patients were asymptomatic with candidiasis urine. Candida and bacterial infections often occur simultaneously. Candida infections of the kidney, mostly secondary, are caused by the spread of blood Candida. Renal cortex and medulla abscesses can occur, which affect renal function in severe cases. Patients may have low back pain, abdominal pain, fever and chills, accompanied by urgency, urinary frequency, proteinuria and hematuria. Urine tests may find hyphae and fungal spores, culture for Candida is positive. Candida albicans is common, but now there is a growing trend of candida glabrata [27].

Fungal Sepsis

The main pathogen of fungal sepsis is Saccharomyces. Most patients have high fever, often above 39 °C. The thermal type varies, of which intermittent fever or remittent fever is more common. WBC count and neutrophil are usually increased. Disease in patients with fungal sepsis followed by severe hepatitis is deteriorating rapidly, even progressing to shock. Fungal septicemia may invade all the tissues and organs. Involved organs have corresponding performance, such as fungal pneumonia, oral fungal infections, intestinal and urinary tract infections.

Disseminated fungal infection often occurs in severe immunocompromised patients who have long-term use of antimicrobial agents. Candida, Cryptococcus, Aspergillus can disseminate along with blood to all of the organs, such as kidneys, lungs, heart, and liver. The condition is dangerous, which often lead to death in a short term.

2.1.4 Other Infections

In addition to common bacterial and fungal infections, severe hepatitis can also be complicated by other pathogens, such as viruses, tuberculosis, protozoa and others.

2.1.4.1 Virus Infection

CMV (cytomegalovirus, CMV), herpes simplex virus (herpes simplex virus, HSV), or varicella - zoster virus (varicella-herpes and zoster virus, VZV) infections are three of common herpes viruses infections in severe hepatitis. Their common characteristics rely on that once the host is infected, the virus can persist for long periods in the host. When the host immunity is weakened, the virus can re-proliferative, which leads to disease resurgence [48].

HSV infections manifest as perioral or external genital herpes, oral and esophageal mucosa inflammation and ulcers, also viremia which leads to pneumonia and encephalitis. Common clinical symptoms of herpes simplex are mild, only a few people show fatigue, fever and other symptoms. Local manifestations are single or multiple blisters on skin or mucous membrane, with tingling. Due to reduced immunity, skin rashes in patients with severe hepatitis perform as varicella-like rash, vaccination herpes, herpes keratoconjunctivitis and disseminated herpes simplex. Severe herpes usually manifests as herpes simplex virus encephalitis with high mortality. There are fever, headache, mental disorders, coma and other clinical symptoms, often without skin herpes lesions. The sites of infection are commonly in the frontal and temporal lobes. Elevated serum antibodies help confirm the diagnosis.

Cytomegalovirus infections are common in cirrhosis of the liver [49]. Interstitial pneumonia is the most common clinical manifestation in severe hepatitis concurrent with CMV infection. Chest CT examinations mainly perform as diffuse interstitial or alveolar infiltrations, very few case show as nodular shadows, occasionally as pleural effusion [50]. Pulmonary consolidation reminds complicated bacterial or fungal infections. Pathology manifestations show alveolar interstitial edema, with varying degrees of fibrosis, lymphocyte infiltration and epithelial cell proliferation. Blood examination shows leukopenia. Because viral pneumonia shares a certain similarity in clinical manifestations, diagnosis mainly depends on pathologic examination. Pathogenic examinations for CMV often use methods below: (1) detect of CMV inclusion body cells and viral particles: eosinophilic nuclear inclusions giant cells are found in respiratory secretions and bronchoscopy lung biopsy specimens. Respiratory secretions, saliva, urine, cervical secretions, liver or lung biopsy specimens were inoculated to human embryonic fibroblast cell culture medium, where cytomegalovirus was separated. (2) immunological methods: CMV antigen from secretions was detected by fluorescent antibody assay, or ELISA, which is conducive to the early diagnosis. Serum antibodies can also be detected by complement combined experiment, in which acute and convalescent serum antibody titer more than 4 times are positive. (3) molecular biology methods: PCR technology and nucleic acid hybridization, which helps to make distinction between a variety of different subtypes of the virus.

Most of VZV infections in patients with severe hepatitis are due to latent virus reactivated. In patients who have had chickenpox, there is a small amount of virus lurking in the spinal cord dorsal root ganglia or cranial nerve sensory ganglia. When severe hepatitis happened, latent virus is reactivated in ganglia due to decreased immunity. The activated viruses spread along with sensory nerve axons to downstream disposal areas, proliferate and cause shingles. In early time, there is paresthesia, itching, and pain in local skin. And then rashes and herpes break out, chaining into a strip, which distribute in denomination or trunk, unilateral, with duration of about three weeks or several months. Part of patients with severe hepatitis show severe disseminated herpes zoster. Varicella-like rashes appear in a few days, often accompanied by fever, which may be complicated by lung, brain damage with a high mortality rate.

2.1.4.2 Tuberculosis and Non-tuberculosis Mycobacterial Infection

Latent tuberculosis can burst to tuberculosis and extrapulmonary tuberculosis when cellular immune function gets weakened. Under normal host immune function, lymphocytes, macrophages and common Langerhans cell may promote granuloma formation and make infection localized. When host immunity is dysfunctional, tissue reaction is very small or even disappeared, leading to mycobacterium growing rather than formation of granulomas, nor any effective defense against infection. Severe hepatitis patients with M. tuberculosis infection may develop acute miliary tuberculosis that manifest as fever, cough, expectoration, bloody sputum, chest pain, and shortness of breath in addition to deteriorated liver function. Moreover, tuberculosis easily spread in patients with severe hepatitis, with poor anti-tuberculosis efficacy. Common extrapulmonary tuberculosis can be lymphatic tuberculosis, intestinal tuberculosis, bone tuberculosis, renal tuberculosis, epididymal tuberculosis, genitourinary tuberculosis, nervous system tuberculosis, tuberculous meningitis [51]. In patients with severe hepatitis concurrent with tuberculous mycobacterial infections, tuberculin reaction is negative in about half of patients, especially in those applied with glucocorticoid. Diagnosis relies on sputum acid-fast staining or PCR, but the diagnostic yield is not high.

Interferon gamma release assays (IGRAs), including QuantiFERON®-TB Gold In-Tube, and the T-SPOT TB, have been extensively used for the auxiliary diagnosis of tuberculosis infection in adults. IGRAs detect circulating T-cells responsive to specific *Mycobacterium tuberculosis* antigens, which are absent in BCG and other non-tuberculosis mycobacteria, and exhibited similar sensitivity and higher specificity than TST in adults [52, 53]. However, these IGRA tests are also affected by host immune status [54]. In addition, the decision regarding whether to treat LTBI should be dependent not only on IGRAs results but also on clinical histories.

NTM generally causes local wound infections. However, in severe hepatitis patients with impaired immune function, non-tuberculous mycobacteria can invade the lungs, causing tuberculosis-like diseases, but rarely causes hematogenous dissemination. Histological examination of the lesion is mainly characterized by epithelioid cell granulomas and foam cell-like balls of tissue proliferation, detection of non-tuberculous mycobacteria [55].

2.1.4.3 Parasitic Infection

Protozoa and worms, such as Toxoplasma gondii, Giardia lamblia, Cryptosporidium and Stercoralis, can also infect patients with severe hepatitis, especially those with immunosuppression drugs or combined with cancer. Main lesions of Toxoplasmosis manifest as lymphadenopathy, hepatosplenomegaly, encephalitis and pneumonia [56]. Clinical manifestations of Giardia lamblia infection are chronic diarrhea and malabsorption, also fever and cholecystitis [57]. Pathological changes are deformation of small intestine villi and lymphoid hyperplasia. Parasites present in small intestinal surface and gallbladder, and the detection of parasites from the stool and duodenal drainage fluid that is eligible confirmed. Strongyloides stercoralis is a weak virulent worm, there is few clinical stercoralis infection [58]. But this worm

infection in patients with severe hepatitis is a serious threat, even causing death. Clinical manifestations include long-term nausea, vomiting, diarrhea, bloating, intestinal paralysis, dehydration, electrolyte imbalance, edema, weight loss and difficulty breathing in cases with extensive lung lesions. All patients have hypoproteinemia and anemia. Patients with increased eosinophils have good prognosis, whereas eosinophils reduction often is a dangerous signal.

2.1.5 Prophylaxis and Treatment of Complications in AECHB

Varieties of complications, such as system or local infections, coagulopathy, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome, acid-base imbalance and water-electrolyte imbalance, are main causes of HBV-associated mortality during deterioration of liver function of AECHB. Reducing and effective treatments of these complications are still nodules in therapy of severe phase of AECHB.

2.1.5.1 Infection

Infection is one of usual complications of AECHB, which shows 18–52% morbidity in civil China. The most common localities are respiratory system, especially lungs, and abdomen, which shows the incidence of spontaneous bacterial peritonitis (SBP) as much as 49%. Others include urinary tract and bloodstream. Gram-negative bacteria are the predominant causes including aerobic Gram-negative bacilli, Escherichia coli, Klebsiella, Enterococcus and anaerobic Bacteroides fragilis, etc. However in recent years, gram-positive bacterial infections are found increasing in patients with AECHB, including Pneumococcus and other Streptococcus, but Staphylococcus aureus infection is relatively infrequent. Fungal infections are usually secondary to bacterial infection. Candida, Aspergillus, Sporotrichosis, Histoplasmosis and Coccidioidomycosis infection, especially Candida albicans, are most frequent in occurrence. But, infections of Cryptococcus and mucormycosis are relatively rare. Beyond of bacterial and fungal infection, other infectious pathogens in AECHB are virus, Mycobacterium tuberculosis, mycoplasma, chlamydia, parasites and protozoa.

Bacterial Infection

Prophylaxis

Bacterial infection is the most frequent complication in AECHB. Infections are more likely occurred in abdomen, including abdominal cavity, biliary tract, gastrointestinal tract, or other parts like respiratory tract, and urinary tract. Keeping oral and perineal asepsis, unobstructed respiratory tract and urine tract, anti-bedsore care for patients with limited mobility, rational use of antibiotics, avoiding long-term use of broad-spectrum antibiotics, strict controlling usage of glucocorticoid, aseptic practices in invasive operation, parenteral nutrition and protecting the intestinal mucosa are all necessities to prevention of bacterial infection in AECHB.

2.1.5.2 Antibiotic Treatment

Empirical use of antimicrobial agents could be determined by the localities of infection without antibiotic susceptibility testing results. Gram-negative infections are more frequent in peritoneal or biliary tract, in which cephalosporin or quinolone could be the first choice. Penicillin and vancomycin could be considered in pulmonary infection. Azithromycin and quinolone could be adopted in urinary tract infection. Metronidazole or Tinidazole could be used in anaerobic infection. Broad-spectrum antibiotics can be used in serious infection, such as ceftriaxone, cefoperazone, cefepime, and carbapenems, but the secondary infections need to be highly concerned. Initial antibiotics should be adjusted according to antibiotic susceptibility testing results as soon as possible. Non-specific immune enhancer agents, such as thymosin, are well used in treatment of infection during AECHB, but it currently still lack of evidence-based support.

Fungal Infection

Prevention

The incidence of fungal infection ranks secondly in AECHB associated infection. Respiratory tract, gastrointestinal tracts and genitourinary system are the major sites. Keeping wards dry and ventilated can help to reduce environmental fungal growth. Oral and perineal cleaning and regularly dental check are necessary. If oral fungal spots are found, alkaline mouthwash and nystatin with glycerol can be used in treatment. For patients with limited mobility, anti-bedsore care is terribly necessary. Rational use of antibiotics, especially avoiding long-term usage of broad-spectrum antibiotics, can prevent secondary fungal infection. Glucocorticoid should be used strictly according to indication. During invasive procedures, aseptic operations are obligated. Regular check of sputum, lungs, urine and feces will facilitate early diagnosis. Empiric antifungal treatment is generally not recommended, but to patients with AECHB with AIDS, especially with count of peripheral blood $CD4^+$ T cell-less than 200/μL or oropharyngeal candidiasis found, the sulfamethoxazole (SMZ-TMP) should be chosen to prevent Pneumocystis pneumonia. Fluconazole should be considered when moderate amount of Candida albicans has been indicated by sputum culture.

Antifungal Treatment

Empirical treatment. If Candida albicans infection is highly susceptible, the initial treatment choice is fluconazole (200–400 mg/days). But if Aspergillus infection is preferred, amphotericin B liposome, itraconazole, caspofungin or voriconazole could be considered as the initial treatment, in which process liver and kidney function should be intensively monitored. To patients with cryptococcal encephalopathy combining severe liver damage, fluconazole or flucytosine combined with intrathecal injection of amphotericin B treatment could be implemented.

Evidence based treatment. Initial antifungal strategy should be adjusted according to antibiotic susceptibility testing results. For certain invasive pulmonary

aspergillosis, combination treatment of several different types of antifungal agents should be considered under monitoring of liver and kidney function. However, for pulmonary mucormycosis infection, the combination of amphotericin B and flucytosine is the only effective strategy.

Amphotericin B

Amphotericin B is a type of polyene antifungals, mainly for Aspergillus, Candida, Cryptococcus, Histoplasma infection, but is invalid in aspergillus terreus or ringworm fungus infection. Intravenous or intrathecal injection of Amphotericin B should be administrated because of non-digestive absorbance. The recommended initial dose of intravenous administration is 1–5 mg/days, and gradually increases to 0.5–1 mg/kg.days. The infusion track needs to be dark and process needs not less than 6 h. The initial dose of intrathecal injection is 0.1 mg/days and gradually increases to 0.5–1 mg/days. Amphotericin B has an extra toxicity to liver and kidney, and also causing hypokalemia. Intensive monitoring of liver and kidney function and serum potassium levels is necessary during treatment, and be sure largely avoiding combination with other agents with liver and kidney toxicity.

Itraconazole

Itraconazole is one of triazole antifungal agents, mainly for Aspergillus, Candida, Cryptococcus and Histoplasma infection, but it is invalid in fungi Fusarium or Zygomycetes infection. The intravenous dose for the initial 2 days is 400 mg/days, administrated by twice, and then followed by 200 mg/days for 2 weeks. The oral doses of 200 mg bid could be subsequent. The total curative course could be determined by the improvement of symptoms and largely absorption of radiographic lesions of infection. The monitoring of liver function is necessary and recommended, especially in long duration treatment. Furthermore, combination treatment with other hepatotoxic drugs should be avoided.

Fluorocytosine

Fluorocytosine is one of bacteriostatics, mainly for Cryptococcosis and Candida infection, frequently based on the combination with amphotericin B. The daily dose for adults is 2.5 g with intravenous dripping speed of 4–10 ml/min. A half dose should be conducted in renal insufficiency. The inhibited administration includes severe liver or kidney dysfunction and allergy to Fluorocytosine, and also cautious treatment for pregnant or breastfeeding women. No combination treatment of Fluorocytosine with Cytarabine or bone marrow suppression agents is recommended.

Fluconazole

Fluconazole is a triazole antifungal, mainly for Candida albicans or Cryptococcus infection, but not as good in Candida glabrata infection, totally invalid in Aspergillus or Candida krusei infection. The recommended daily dose for adult is 200–400 mg, and the initial dose should be doubled.

Voriconazole
Voriconazole belongs to triazole antifungals, mainly for Candida, Cryptococcus, Aspergillus, Fusarium and Histoplasma capsulatum infection, especially used for invasive aspergillosis and invasive fluconazole-resistant Candida infection, but is invalid for Mucor or Rhizopus infection. The recommended intravenous initial dose in adult is 6 mg/kg, q12h, with infusion rate of 3 mg/kg within 1–2 h. The maintenance dose from the second day is 4 mg/kg, q12h. To patients without tolerance to normal dose, it could be reduced to 3 mg/kg, q12h. The reduction of daily dose could not be necessary in mild to moderate liver dysfunction. But intravenous administration should be avoided in patients with severe renal dysfunction. The side effects of Voriconazole include transient visual disturbances, mental disorders, thrombocytopenia and so on.

Caspofungin
Caspofungin belongs to echinocandin antifungals with antibiotic spectrum of pathogenic Aspergillus, Candida and Pneumocystis, without in Cryptococcus neoformans, Fusarium and Mucor. It is mainly used for invasive aspergillosis. The initial dose for adults is 70 mgqd, and with subsequent 50 mg qd. The infusion time is no less than 1 h. No fixation curative course is suggested. Caspofungin should be avoided for patients with severe liver function, because of highly hepatic distribution and metabolic pathways during treatment.

2.2 Section 2: Coagulopathy

Chuan-Long Zhu

Liver is the largest solid organ in the body, and it plays a central role in the clotting process, except for general function such as metabolism, detoxification and choleresis [59]. A majority of the coagulation factors are synthesized almost exclusively in the liver. In the pathogenesis of severe hepatitis, massive hepatic necrosis leading to reduced production and dysfunction of coagulation factor. In addition, the increased levels of anticoagulation and platelet abnormalities also contribute to occurrence of coagulopathy, which were further exacerbated by severe complications such as spontaneous bacterial peritonitis (SBP) and hepatorenal syndrome (HRS). Coagulation abnormalities, even disseminated intravascular coagulation (DIC) often occur in those patients with severe hepatitis. Therefore, the changes in coagulation factors were usually used to evaluate prognosis of severe hepatitis [60].

2.2.1 Mechanisms

2.2.1.1 The Liver and Physiology of Blood Coagulation
The normal procedure of coagulation includes two independent coagulation process, namely intrinsic and extrinsic pathway, which initiated by coagulation factor

XII, and factor VIIa/tissue factor, respectively. The two pathways reach a confluence at the points of factor Xa and Va. Wherein Factor Xa activates prothrombin to thrombin in the presence of Ca^{2+} and factor Va bound to membrane surfaces, and then thrombin converts fibrinogen to fibrin. Thus, blood becomes clotted and the "Y" shaped pathway was established [61]. Simultaneously, there are some anticoagulation systems existing in our body, which prevents the formation of thrombus, then keeps normal circulating of blood flow. It also participates in maintaining of normal permeability of the blood vessel [62]. Among anticoagulation systems, the most important one is the fibrinolytic system, whose basic process can divided into two stages, i.e. plasminogen activation and fibrin degradation [63]. Plasminogen activators include tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), and the principal function of these two plasminogen activators is to convert plasminogen to plasmin, then plasmin cleaves fibrin into soluble small peptides, namely fibrin degradation products [64]. Moreover, the process of fibrinolysis was affected by fibrinolysis inhibitors, such as plasminogen activator inhibitor (PAI) and alpha2antiplasmin (α2-AP). Those fibrinolysis inhibitors can either inhibit plasminogen activation, or reduce the function of plasmin [65]. Thus, there is a dynamic balance between coagulation and anti-coagulation systems under physiological conditions (Fig. 2.1) [66]. Many factors involved in coagulation process, i.e. clotting factors, thrombin inhibitor, fibrinolytic system and so on, are synthesized almost exclusively in the liver. Thus, liver plays a pivotal role in remaining the balance between hemorrhage and coagulation under physiological conditions (Table. 2.1) [67]. It is the important basis for pathogenesis of coagulopathy that massive hepatocyte necrosis occurs in severe hepatitis patients, resulting in reduced production and dysfunction of those blood clotting factors [68].

2.2.1.2 The Mechanism of Coagulopathy in Severe Hepatitis

Abnormal Synthesis of the Coagulation Factors

Coagulation abnormalities, such as decreased production and dysfunction of coagulation factors, are commonly found in severe hepatitis, There are at least 14 types of factors participated in the coagulation process, including 12 classical coagulation factors, namely factor I/fibrinogen, factor II/prothrombin, factor III/tissue factor, factor IV/Calcium ions, as well as factor V, VII, VIII, IX, X, XI, XII, and factor XIII. Some bradykinin factors, such as RK (prekallikrein) and HMWK (high molecular weight kininogen) are also associated with coagulation. Among above factors, the others belong to proteins excepting factor IV. Plasma coagulation factors are synthesized exclusively in the liver, excepting factor III/tissue factor, factor IV, factor VI/activated factor V, factor VIII and factor VIIIa [69].

Massive hepatic necrosis leads to the decreased production of clotting factor in patients with severe chronic hepatitis. Moreover, the coagulation factors are sensitive indicators for clinical evaluation of severe hepatitis. It is common that the serum levels of factor V, VII, IX, X, XI and prothrombin decreased in the patients with severe hepatitis. On the contrary, clotting factor VIII is synthesized, and then excreted increasingly by the mononuclear phagocytic cells with stimulation of

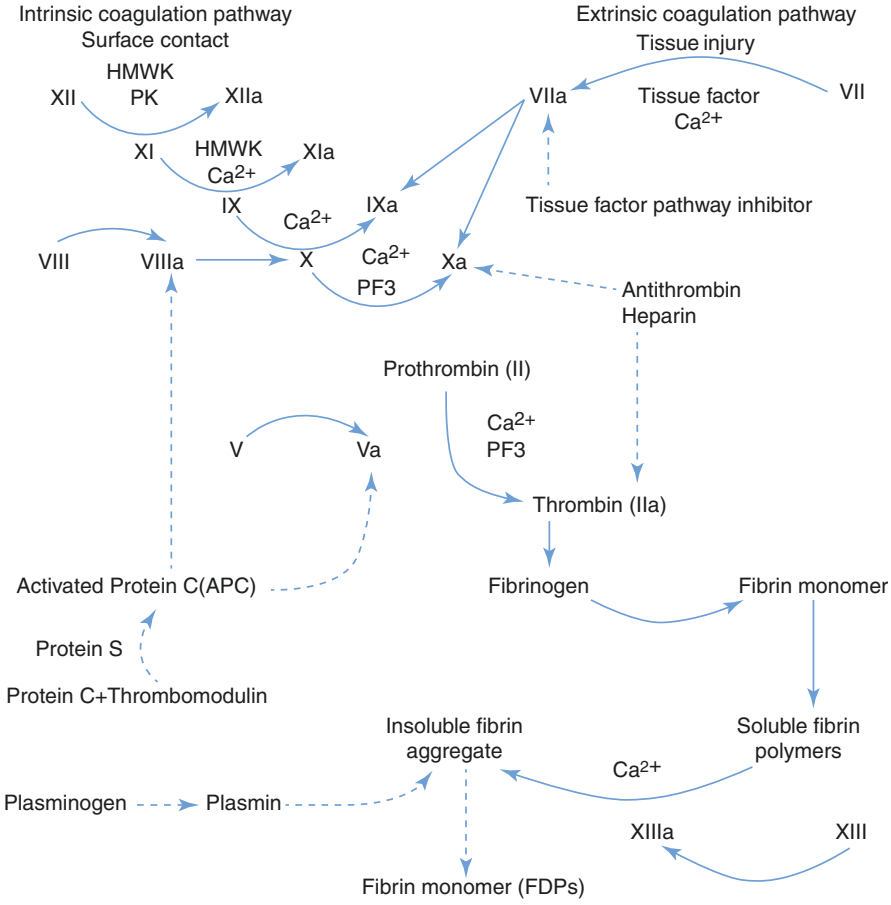

Fig. 2.1 Mechanism of normal clotting process (solid-light arrows) and anticoagulant system (dashed arrows)

various inflammatory cytokines in the patients with severe hepatitis [70]. Anorexia and antibiotics overuse lead to obstacles in assimilation, absorption, and application of vitamin K in these patients. Vitamin K dependent coagulation factors includes factor II, VII, IX, and X. The function of γ-hydroxylation was weaken by the deficiency of vitamin K and damage of hydroxylase, then the abnormal clotting factors without γ-hydroxy glutamate were synthesized. Finally, these abnormal clotting factors lead to dysfunction of the Vitamin K dependent coagulation factors [71]. Coagulation factor VII eliminated significantly in the initial stage of liver injury was usually used as an early diagnosis index [72]. In addition, coagulation factors II and X, then factor IX decreased markedly, along with exacerbation of liver injury. The deficiency of Vitamin K induced by obstructive jaundice, malabsorption syndrome,

Table 2.1 Primary characteristics of factors participating in coagulation process

Factor	Fibrinogen	Production sites (Need vitamin K or not)	Participation in the coagulation process
I	Fibrinogen	Liver (No)	Common
II	Prothrombin	Liver (Need)	Common
III	Tissue factor	Organization, Endothelial, Monocytes (No)	Exogenous
IV	Calcium (Ca2+)	\	Common
V	Labile factor (Proaccelerin)	Liver (No)	Common
VI	Activated factor V	\	Common
VII	Stabilizing factor (Transition acceleration factor precursor)	Liver (Need)	Exogenous
VII	Antihemophilic globulin	Liver, Endothelial Cells (No)	Exogenous
IX	Plasma thromboplastin component (Christmas factor)	Liver (Need)	Exogenous
X	Autologous prothrombin C (Stuart-Power factor)	Liver (Need)	Common
XI	Plasma thromboplastin precursor	Liver (No)	Exogenous
XII	Surface factor (Hageman factor)	Liver (No)	Exogenous
XIII	Fibrin-stabilizing factor	Marrow (No)	Common
PK	Prekallikrein	Liver (No)	Exogenous
HMWK	High-molecular-weight kininogen	Liver (No)	Exogenous

can be rescued after intravenous injection of vitamin K, which is different from coagulopathy caused by liver injury.

The plasma level of fibrinogen is within normal range in patients with compensatory cirrhosis. However, patients with severe hepatitis or liver failure have a significant reduction in the level of fibrinogen, and then develop dysfibrinogenemia [73]. Fibrinogen, as the main hydrolysis substrate of thrombin, is the crucial factor in the coagulation process. Thus, decreased levels or abnormal structure of fibrinogen leads to coagulation abnormalities in patients with severe hepatitis [74]. There are two clotting factors associated with thrombin except for fibrinogen, i.e. Factor V and factor XIII. Factor XIII induce a crosslink of SFM (soluble fibrin monomer), resulting in the formation of insoluble fibrin [75]. It reports that the levels of circulating coagulation factor XIII were decreased in 30% of patients with liver disease, resulting in fibrin decline or abnormality. These patients with liver disease will receive a bad prognosis if the plasma level of fibrin was at level below 35% of normal concentration [76]. It is uncommon that the plasma level of plasma factor V decreased markedly in patients with severe hepatitis, except for those complicated with DIC or hyperfibrinolysis. The low level of factor V cannot induce the formation of the enzyme-substrate complex, which delay the activation of prothrombin [77].

Contact factors include classical coagulation factor XII and XI, as well as some bradykinin factors, i.e. PK and HMWK. These factors contact with vascular intima damage or abnormal surface in blood vessel walls, then active the intrinsic coagulation pathway [78]. Moreover, the contact factors can connect with bradykinin, fibrinolysis and complement system. In addition, coagulation factor XIII also activates fibrinolysis system as the initiating factor. Factor XII deficiency does not cause excess bleeding, but induce thrombus due to its decreasing activation of fibrinolytic system [79].

Abnormal Synthesis of the Anti-coagulation Factors

There is a dynamic balance between pro- and anti-coagulation systems in physiological conditions, which keep normal blood circulation in the body. The anti-coagulation system includes physiological anti-coagulation factors (e.g. antiprothrombin-III, protein C, and so on) and fibrinolysis factors (e.g. plasminogen, α-2 plasmin inhibitor, and so on) [80].

AT-III, with a half-life period of 2.8 days, synthesized mainly in hepatocytes and partly in endotheliocyte, is responsible for about 70% of anticoagulation in plasma. It is the main reason for low plasma level of AT-III that decreased production and increased consumption of AT-III caused by hepatocytes necrosis in patients with severe hepatitis. The activity of AT-III obviously decreased in severe hepatitis [81]. Therefore, heparin, thrombin, activated coagulation factors (e.g. factor X, IX, XI, XII) cannot be inhibited, due to rare AT-III combining with them [82]. There is a negative correlation between AT-III: A (the activity of AT-III) and PT (Prothrombin time), which indicate that the level of AT-III: A decreased obviously along with exacerbation of liver injury [83]. The plasma levels of pro- and anti-coagulant factors are low in patients with liver injury. When the necrotic tissue and cytolysis are released in the blood, the balance of pro- and anti-coagulant is destroyed. Finally the depletion of AT-III by massive activated coagulation factors will lead to DIC [84].

Massive hepatocytes necrosis, Vitamin K deficiency, and protein C without γ-Hydroxyglutamic Acid result in blocking activation of protein C. Thus, Va, VIIIa and PAI cannot be degraded, and the plasma level of them will rise up, due to reduction of activated protein C (APC) in the patients with severe hepatitis [85].

Plasminogen synthesis will decreased by about 70% in the liver when sever hepatitis occurs. As the activators of plasminogen, tPA and uPA are synthesized by vascular endothelial cells, and their production will increase after vascular endothelial cells initiated with virus, immunocomplex or endotoxin in the patients with severe hepatitis B [86]. With exacerbation of liver injury, PAI synthesis decreased significantly in the liver. The main physiological activity of PAI is to inhibit tPA induced plasminogen activation. Therefore, the activity of tPA will increase with reduction of PAI synthesis, resulting in promoting the conversion of plasminogen into plasmin [87]. Plasmin, as a kind of powerful proteolytic enzyme, can hydrolyse fibrinogen into FDPs, degrade coagulation factors, and inhibit platelet aggregation, resulting in the aggravation of bleeding [88].

The Depletion of Coagulation and Anticoagulation Factors

Hyperfibrinolysis can be caused by congenital or acquired reason, and it commonly leads to a rapid depletion of coagulation and anticoagulation factors, especially in those patients with DIC [89]. Synthesis and secretion of tPA and uPA markedly increase, while PAI, namely tPA or uPA inhibitor, has been a decrease in the plasma of severe hepatitis patients, resulting in hyperfibrinolysis [90]. At the same time, mononuclear phagocyte system cannot degrade plasminogen activator, also leading to hyperfibrinolysis. It is not necessarily accompanied by hemorrhagic tendency, although there are many risk factors that can cause hyperfibrinolysis, even hyperfibrinolysis occurred in severe hepatitis [91]. In addition, FDPs inhibit fibrin monomer polymerize function, and also block platelet aggregation, then further worse the deficiency of hemostasis and coagulation, finally leading to the aggravation of bleeding tendency [92].

Increased Anticoagulant Substance in Blood Circulation

Studies have shown that low-level endogenous small molecule heparin in patients with chronic hepatitis may be associated with many factors, such as increased mastocyte, decreased production of heparinase in the liver and reduced activity of PH4 (platelet factor4) [93]. When the disease progress to cirrhosis or chronic severe hepatitis, PT was significantly prolonged, while endogenous heparin wasn't increased markedly, indicating that low-level endogenous heparin has little effect on PT elongation and hemorrhagic tendency [94]. However, if the patient is undergoing the following condition together, the endogenous small molecule heparin will increase significantly. (1) Esophageal variceal bleeding with serious infections (such as abdominal infection, biliary tract infection and pulmonary infection) or portal hypertension. (2) Combining with the significant increased white blood cell count. (3) Percentage of neutrophils >75%. The level of endogenous small molecule heparin in plasmin will decrease after those infections were cured. Taken together, these data indicate that increased level of endogenous small molecule heparin in blood circulation was closely related to severe hemorrhagic tendency when combining with infection [95, 96].

Platelet Abnormality

Platelet significantly promotes blood coagulation through connecting with many coagulation factors (such as fibrinogen, factor V, factor XI, factor XIII and so on). α-granule includes fibrinogen, factor XIII, and some platelet factor such as platelet factor2 (PF2) and platelet factor3 (PF3), which promote coagulation activation process of factor XII and factor XI can be accelerated by activated platelets [97]. It is estimated that PF3 provided by platelets could accelerate activating of thrombin by twenty thousand times. Xa and factor V could not be inhibited by AT-III and heparin if they were linked with PF3. When platelets aggregated and formed platelet plugs, the process of coagulation was initiated at this site, and then platelets reveal a large number of phospholipid surfaces, which were helpful for activating of factor X and fibrinogen [98]. Various platelet factors were released from α-granule after platelet aggregating, and then hemostyptic fibre was produced increasingly. Those

hemostyptic fibre finally produced blood clot formation after capturing the other hemocytes. Thus, platelet plugs would progress independently of platelet disintegrating gradually [99].

Two aspects of platelet abnormalities consist of depletion and dysfunction. In patients with chronic hepatitis and cirrhosis, the occurrence of decreased platelet level is usual through hypersplenism accompanied with other hemocyte decreasing [100]. The ratio of which reaches 37–77%. Its incidence rate in severe hepatitis and explosive hepatic failure also reaches approximately by 50%. When severe hepatitis occur, myelosuppression, decreased megacaryocyte replication leading short-life platelet, lacking of hemopoietic material such as Vitamin B, folate and so on, all of these can initiate thrombocytopenia [101]. Other reasons leading to thrombocytopenia contain low expression and metabolic disturbance of thrombopoietin (TPO), as well as platelet antibody production. Researches show that the serum level of TPO related positively to platelet expression [102]. We next studied platelet associated immunoglobulin (PAIg) and its sorts such as PAIgG, PAIgA, PAIgM etc., with chronic hepatitis. In line with previous reports, we found that serum levels of PAIg and PAIgG negatively correlated with blood platelet count, corroborating the crucial role of the PAIg-mediated autoimmunization in controlling thrombocytopenia in viral hepatitis [103].

Various factors impact platelet function forwardly or passively. Increased expression of oxide and prostacyclin, two kinds of platelet inhibitor derived by endothelium, may inhibit platelet activation in vivo [104]. On the other hand, increased serum level of von Willebrand factor (vWF) can promote platelet adhering and aggregation in patients with cirrhosis. When severe hepatitis occur, 66.7% and 77.8% of patients have decreased levels of platelet adhesion rate and platelet aggregation rate (PAR), respectively. In addition, reduced effectiveness of PF3 and abnormal clot contraction occur in patients with severe hepatitis by an incidence of 65.6% and 77.8%, severally [105].

Potential Risk Factors for Aggravating Bleeding

Patients with terminal liver disease significantly exert hemorrhagic tendency, especially in the digestive tract [106]. The latest report indicated that basic laboratory examinations for coagulation function testing in common use at present, such as PT, APTT, international normalized ratio (INR) etc., have little correlation with occurrence of gastrointestinal bleeding in these patients, thereby revealing the importance to search and pay close attention to those complicating disease upregulating bleeding risk, such as bacterial infection, renal failure, hemodynamic change after portal hypertension, dysfunction of endotheliocyte as well as macrophagocyte and so on [107].

It is common to see renal failure occurrence in advanced hepatopathy. When it happened, acquired platelet dysfunction, abnormal activation of platelet and vascular wall, anemia and so on, all of them significantly promote hemorrhage [108]. As another severe complication, bacterial infection is also very important and common [109]. When tumor necrosis factor (TNF) were injected into healthy individuals, we found that endotoxin have an important role to exert its function directly in clotting

cascade reaction. Early researches indicated that the body can express endogenous heparin-like substance through stimulation by endotoxin in patients with cirrhosis [110]. Furthermore, some studies revealed the relevance of endotoxin with prothrombin fragment, indicating that infection promoted occurrence of DIC-like status in laboratory examination in cirrhosis [111].

Coagulation system became weaker in patients with chronic hepatitis. It can hardly mediate factors due to the relative deficiency of pro- and anticoagulation factors. When the balance was destroyed, it may tend to hemorrhage or thrombosis depending on related risk factors which were in the ascendant.

Hypercoagulability in Chronic Liver Disease

To assess abnormality of blood coagulation in patients with liver disease, we should be in consideration of hypercoagulability, one state may easily be overlooked. Otherwise, it will be unfair. Current literatures indicated that, unlike previous concept that the body of patients initiated anticoagulation state automatically when infected by the hepatitis virus. Various clinical evidences revealed that hepatitis virus infecting cannot inhibit thrombus forming. Furthermore, it may increase dangerous to thrombus forming especially in the portal system, for existence of individuals having hereditary mutation to promote thrombus forming [112].

Slower bloodstream, abnormal fibrinolysis initiated by blood stasis, and decreased activity of anticoagulant accelerate formation of venous thrombosis. Moreover, the changes of platelet phospholipids membrane activity were also helpful to the formation of thrombosis in patients with chronic liver disease [113]. It is reported that the incidence of periphery deep venous thrombosis and pulmonary embolism was 0.5 and 1.0% in patients with cirrhosis, respectively [114]. The rate of portal vein thrombosis is about 1% in patients with compensated cirrhosis, but it reaches 8–25% in the candidates waiting for liver transplantation [115]. For mutations (such as Leiden mutation of factor V, G20210A mutation of prothrombin, C677 mutation of methylenetetrahydrofolate reductase) or the existence of antiphospholipid antibody syndrome, the hypercoagulable state will be represented in patients with liver disease [116].

The hypercoagulable state represents diverse modes in the body of patients with chronic hepatitis. Among them, thrombosis is more traditional and common.

2.2.1.3 Mechanism of DIC Complicated with Severe Hepatitis

The mechanism of DIC complicated with severe hepatitis may contain several aspects as follows.

1. With attacks from endotoxin, virus and immune complex etc., endotheliocyte was injured, and then it activated plasmakinin system and complement system, leading to the aggravation and participation of DIC progress. Damaged endotheliocyte can simultaneously activate factor VII, intrinsic coagulation pathway and platelets, and participate in micro-thrombosis with platelets adhesion and aggregation beneath endothelium [117, 118].

2. In patients with severe hepatitis, massive necrotic hepatocytes activated extrinsic coagulation system with the releasing of various tissue thromboplastin-like substances [119].
3. Hepatocellular necrosis or dysfunction decreased expression of anti-coagulation factors such as AT-III, PC, protein S and so on, and then it enhanced activation of thrombin and plasmin [120, 121].
4. Impaired function of the mononuclear phagocyte system. Mononuclear phagocytes can express activated tissue factor with the releasing of TNF, IL-1 and platelet activating factor (PAF) on its surface after stimulation by endotoxin, inflammatory cytokines and complement activation. TNF and IL-1 can decrease the activation of protein C through its function to increase the expression of plasminogen activator and plasminogen activator inhibitor-1 and to inhibit the production of thrombomodulin (TM) [122]. Activated clotting factor and other factors with promoting coagulation can lead to the occurrence and aggravation of DIC, since it cannot be promptly removed [123].
5. The onset and enhancement of fibrinolysis. Massive clotting factors and platelets were depleted in the extensive formation in vivo microthrombus. Thrombin promoted the conversion of fibrinogen into fibrous protein [124]. Simultaneously, it activated fragments which dropped in the formation of activated factor XIII, factor Xa and factor XIIa. All of them can activate plasmin, and it enhanced fibrinolysis with tPA, one factor released by damaged blood vessel endothelium [125]. Fibrinogen and fibrous protein were degraded after plasminogen activation to generate the corresponding FDPs, one factor inhibit blood coagulation and also block platelet aggregation [126]. Taken together, the above process exacerbates bleeding initiated by coagulation factors depletion and platelet deficiency.

2.2.2 Clinical Manifestations

2.2.2.1 General Clinical Manifestations

Coagulopathy, characterized by prolongation of blood clotting time, tendency of hemorrhage, or even DIC, were commonly found in severe hepatitis patients. It may cause uncontrolled external or internal hemorrhage. As common clinical symptoms, the external hemorrhages include gingivale or nasal mucosal bleeding, skin petechiae, the punctures or injection-site ecchymosis, and so on. The internal hemorrhages include esophagogastric varices, intracranial, subcutaneous, and muscle interval bleeding. Except for esophagogastric varices hemorrhage, the other internal hemorrhages rarely occur in those patients. In addition, massive hemorrhage from esophagogastric varices is a serious medical emergency, can potentially cause death and cardiac arrest if proper medical treatment is not received quickly [127].

2.2.2.2 Clinical Manifestations of Severe Hepatitis Complicated with DIC

When DIC occur, a wide range of thrombogenesis in microvasculature can cause circulatory collapse, characterized by low blood pressure and shock. Microcirculatory

dysfunction occur after microthrombosis producing, resulting in hypofunction of multiple organs (e.g. kidney, liver, lung and pancreas), which perform from dysfunction to failure, with illness progressing [88]. Crushed by the fibrous protein in the vessels, red blood cell destruction can lead to intravascular hemolysis. At the early stage of disease, it shows hypercoagulability. The blood in the needle is easy to coagulate, when getting blood sampling from the vein. After that, it comes with the stage of consumed hypocoagulation. The consuming of a great number of blood coagulation factors leads to significant tendency of hemorrhage [107]. It is difficult to stop bleeding in many parts of the body, including visceral organs, operative sites, injection sites, puncture sites, and mini-invasiva sites [128]. When the third stage, namely secondary fibrinolytic stage comes, a great number of the blood coagulation factors have already been used up, resulting in severe bleeding under the condition of low coagulation. Shock, acidosis and MODS make the patient's condition continue deteriorating, and are also the main reasons for death [129].

2.2.2.3 Clinical Manifestations of Severe Hepatitis Complicated with Thrombosis

The latest study indicates that thrombosis is also a noticeable state in cirrhosis and end-stage liver disease. Portal thrombosis and peripheral vein thrombosis (e.g. deep vein thrombosis and lung embolism) are commonly seen. The deep vein thrombosis is more danger than lung embolism. Anyway, the incidence rate of thrombosis in cirrhosis and end-stage liver disease is still very low. The clinical symptoms depend on the embolizing position after the thrombosis occurs [130].

2.2.3 Laboratory Tests

2.2.3.1 Blood Clotting factors

Presently blood clotting factors test is the most maturely and frequently used test in the term of blood coagulation function in the world. The indicators including prothrombin time (PT), international normalized ratio (INR) and prothrombin time activity (PTA) have always been chosen in the patients with severe hepatitis.

Prothrombin Time (PT)

Prothrombin time (PT) reflects whether there are anticoagulant substances in the extrinsic blood coagulation system and blood circulation or not. The elongation of prothrombin time (PT) presents the declined activity of several blood clotting factors including factorII (FII), factorV (FV), factor VII (FVII), factorX (FX) or the existence of anticoagulant substances. In severe hepatitis patients, the incidence rate of prothrombin time (PT) elongated can reaches to 90%, thus it is regarded as a sensitive and frequently used indicator in the term of liver function [131]. Another index international normalized ratio (INR), a reckoned ratio calculated from prothrombin time (PT) and international sensitivity index (ISI), making prothrombin time (PT) between different laboratories and different reagents comparable, is an international general indicator. The guides about acute—on—chronic liver failure

and acute liver failure take the index, INR ≥ 1.5, as one of the most significant diagnostic criteria in the American Association for the Study of Liver Diseases (AASLD) and the Asian Pacific Association for the Study of Liver (APASL). International normalized ratio (INR) can also be used as a monitor on blood coagulation function [132].

Hepaplastin Test (HPT)

Hepaplastin test (HPT), reflecting not only blood coagulation mechanism in hepatitis patients but also the function of hepatocytes to synthetize vitamin K dependent clotting factors including factorII (FII), factor VII (FVII), factorIX (FIX), factorX (FX) synthetically, is a test about liver reserve function; but this indicator can't reflect the change of factorV (FV). When severe hepatitis occur, the function of liver to synthetize above-mentioned clotting factors declines and the time of hepaplastin test (HPT) elongates. With illness progressing, HPT continues elongating. Survivors undergoing effective therapies can have gradual recovery in the time of hepaplastin test (HPT). So this test is regarded as the specific test of liver diseases or the optimal indicator reflecting liver reserve function [133].

Prothrombin Time Activity (PTA)

The severity of liver damage is positively correlated with the decline degree of prothrombin time activity (PTA): the more severe damage occurs in hepatocytes, the more significantly prothrombin time activity (PTA) will decline. Therefore prothrombin time activity (PTA) <40% and total bilirubin (TBil)>171 μmol/L have always been used as the main laboratory indicators to diagnose severe hepatitis domestically. PTA <40% is also regarded as diagnostic criterion of blood coagulation dysfunction in the guideline of acute-on-chronic liver failure in the Asian Pacific Association for the Study of Liver (APASL) [134]. In severe hepatitis, total bilirubin (TBil), total cholesterol, prothrombin time activity (PTA) and complications (e.g. rectory hyponatremia, hepatic encephalopathy, hepatorenal syndromes and so on) are all independent risk factors to evaluate prognosis. The lack of any factors including factorI (FI), factorII (FII), factorV (FV), factorVII (FVII), factor X (FX), can lead to the decline of prothrombin time activity (PTA). Moreover the half-life time of those factors are extremely short, factorII (FII) 50 –80 h, factorV (FV) 12 –24 h, factorV (FV) 2 –6 h, factorV (FV) 48–60 h, respectively. It means that when hepatocytes suffer from severe serious damage and necrosis in severe hepatitis, prothrombin time activity (PTA) will have dramatic decline just in a few days. As a result, prothrombin time activity (PTA), characterized by significant advantages in evaluating patients condition and judging prognosis over other laboratory indicators, has been widely used.

Activated Partial Thromboplastin Time (APTT)

Elongation of APTT prompts the lack of any clotting factor belonging to intrinsic coagulation system or the existence of anticoagulant substances. The incidence of the elongation of APTT reaches to 80–100% in severe hepatitis patients [135].

The Other Tests

FXII:C reflects liver synthetic function. FVII, characterized by the shortest half-time 6–8 h, is the first one to be affected when facing liver synthetic dysfunction. On the contrary, FV:C, characterized by a relatively long half-time, is one of the latest factors to be affected and is correlated with the degree of liver damage. It prompts severe liver failure, bad prognosis and even easy death when plasma levels of FV:C under 20%. Some literatures report that FV:C and PTA can be used as significant prognostic factors in liver failure and significant screening indicators in liver transplantation. However, the indicators- FXII:C and FV:C- are not listed as routine examination items. They are just selected on the condition of illness demand [136, 137].

2.2.3.2 Anti-clotting Factors

The main test about anti-clotting factors is the determination of antithrombin III activity (AT-III:A). In the state of pathosis, the decline of antithrombin III activity (AT-III:A) is not parallel with the decline of antithrombin III (AT-III) content, namely the depletion of antithrombin III activity (AT-III:A) more apparent. Owe to this, it has more clinical value to determine antithrombin III activity (AT-III:A) rather than antithrombin III (AT-III) content. Moreover, anti-clotting factors tests include the determination of protein C activity (PC:A) as well [138].

2.2.3.3 Fibrinolysis

Serum levels of FDPs are very low in normal people. Significantly elevating of FDPs indicates the existence of hyperfibrinolysis and reflects the occurring of DIC indirectly. There are many assay methods including immunization Fi test (namely latex particle agglutination test, normal titer<1:8), FDPs flocculation test, radial immunodiffusion staphylococcal clumping test, indirect hemagglutination inhibition test, enzyme-linked immunosorbent assay (ELISA) and so on. If the serum levels of FDPs elevate, it indicates acute DIC may occur [92].

Plasma protamine paracoagulation test (3P test) and ethanol gel test (EGT) reflect the soluble fibrin complexes in plasma. Soluble fibrin complexes, combination of FDPs and fibrin monomer, can't be solidified by thrombase. But protamine is able to make the complexes isolate and then fibrin monomers separate out again. The paracoagulation test means self-polymerization between fibrin monomers and FDPs, then forming macroscopic flocks. Ethanol gel test (EGT) has the same principle with Plasma protamine paracoagulation test (3P test), whereas the former has a lower positive rate. The two methods may have false negative results and false positive results. In contrast, EGT has a relatively lower sensitivity, while 3P test has a relatively lower specificity. For example, relative small molecular mass of the shreds of FDPs may lead to negative result using 3P test. So it is more valuable to compare the two indicators simultaneously [139, 140].

Euglobulin, a protein (including fibrinogen, plasminogen and other activins except for fibrinolysis inhibitor) separating out from plasma in acid circumstances, can be used to determine whether levels of plasminogen activators increase or not [141]. When hyperfibrinolysis occurs, plasma levels of plasminogen decline, plasma

levels of plasmin increase, and euglobulin suffer from accelerated dissolution by a great number of plasmin. The normal value of euglobulin lysis time (ELT) is above 2 h. That is to say dissolution within 2 h means the occurrence of hyperfibrinolysis. Domestic population data report the positive rate of ELT test reaches 25–42.9%, when DIC occur [142].

Furthermore there are other tests about fibrinolysis including tissue-type plasminogen activator test (t-PA), plasminogen activator inhibitor test (PAI), plasminogen antigen test (PLG:Ag), plasmin activity test (PL:A), α2-plasmin inhibitor test (α2-PI) and so on [143, 144].

2.2.3.4 Platelet Quality and Quantity

Blood platelet count reflects the absolute number of platelet in peripheral blood circulation. According to the reports at home and abroad, platelet count have a significant decline in patients with chronic severe hepatitis. Moreover, studies on domestic population find that platelet count ranges from 68×10^9/L to 130×10^9/L in peripheral blood of severe hepatitis patients. Some studies have already compared the platelet count among early stage, typical stage and late stage in severe hepatitis, and they have turned out to be 130×10^9/L, 109×10^9/L and 87×10^9/L respectively. As a result, it indicates that platelet count is positively correlated with the severity of hepatitis [145]. Except for platelet count, other routine indicators including mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) also have significant reference value. When severe hepatitis occurs, the above-mentioned three indicators will be dramatically lower, and they have the tendency to continue declining with illness progressing.

What's more, platelet quality tests include platelet aggregation rate, platelet factor 3 validity tests and blood clot retraction test (Table 2.2) [146].

2.2.3.5 Laboratory Tests for DIC

Laboratory Tests for Hypercoagulability

Diagnosis of Blood hypercoagulability in the early stage of DIC relies on several molecular marks including plasma thrombinogen segment 1 + 2 (F1 + 2), thrombin-antithrombin complex (TAT) and D-dimer, due to no significant changes in general laboratory tests. This stage is characterized by the elevated levels of those three molecular marks, and levels will increase more significantly with the occurrence of typical DIC symptoms. Dynamic monitoring of above-mentioned indicators is helpful for early diagnosis of DIC [147].

Tests for Hypocoagulability and Secondary Fibrinolysis

The stages are mainly characterized by the decline of blood clotting factors (including factorV (FV), factorVII (FVII), factorXII (FXII), factorIX (FIX), factorX (FX)), platelet count and plasminogen, and increasing of fibrinolytic

Table 2.2 the main laboratory tests and the positive rate of them in severe hepatitis patients with blood coagulopathy

Name	Changes	Normal reference value	Positive rate when severe hepatitis occurring (%)
1. Tests about clotting factors			
PT	Lengthen	11–13 s	63–71
APTT	>10s	(37 ± 3.3)s	82–85
INR	–	1.0 ± 0.1	>80
HPT	Decrease	90.1% ± 13.4%	93
Fz	Decrease	2–4 g/L	16–20
Fγ:C	Increase	(103 ± 26)IU/L	100
TT	>3 s	16–18 s	–
2. Tests about anti-clotting factors			
AT-β:A	Decrease	108.5 ± 5.3%	100
PC:A	Decrease	64–147%	84
3. Tests about fibrinolysis			
tPA	Increase	(0.3–0.6) IU/mL	92
PAI	Decrease	(0.1 ± 1.0)Au/mL	76
PLG:Ag	Decrease	(0.22 ± 0.03)g/L	56
PL;A	Fibrinolysis: increase Hypercoagulability: Decrease	85.5% ± 27.83%	–
a2-PI	Decrease	0.8–1.2 inhibitory units/mL	55
3P test	–	Negative	negative
ELT	Shorten (<70 min)	Calcium addition method (129.8 ± 41.1)min Enzymolysis method (157.5 ± 59.1) min	–
FDPs	Decrease	urine<10 mg/L	85–90
D-Dimer	Increase	Negative (latex agglutination test) 0–0.256 mg/L (ELISA)	9–15
4. Test about platelets quality and quantity			
PLT	Decrease	(100–300) × 109/L	–
PAR	Decrease	MAR (maximum aggregation rate) ADP (1.0 mmol/L)62.7% ± 16.1% ADP (0.5 mmol/L)37.4% ± 14.3% Adrenaline (0.4 mg/L)67.85% ± 17.8%	–
PF3aT	Decrease	The recalcification time of group is under 5 s longer than that of group	–
CRT (whole blood method)	–	Clot retraction rate 65.8% ± 11.0%; Clot retracts partly after 2 h, and retracts completely after 18–24 h	–

activity; The elevating levels of fibrin(−ogen) degradation products (FDPs) and D-dimer; The shortening of euglobulin lysis time (ELT) and the positive reaction of 3P test [148, 149].

With the occurrence of DIC, there will be a wide range of blood coagulation and highly-activated fibrinolysis in the patient's body [150]. What's more, abnormal increased soluble fibrin monomer and FDP fragments will exist in plasma [151]. The level of soluble fibrin monomer complex (SFMC), a complex combined fibrin monomer with FDP, is determined by 3P test. The 3P test shows positive with the occurrence of secondary fibrinolysis, whereas it shows negative with the occurrence of primary fibrinolysis. This means 3P test is negative when there is no blood coagulation. Domestic population data report that the positive rate of 3P tests reach 50–60%. However, the test can't be used as the ideal diagnostic indicator for DIC, owing to many affected factors. False positive reactions are mainly found in the following conditions: gastrointestinal bleeding, massive hemoptysis, malignant carcinoma or blood sampling reserved improperly, whereas false negative reactions are usually found at the late period of the stage of secondary fibrinolysis [152, 153].

As mentioned above, plasma thrombinogen segment 1 + 2 test (F1 + 2), thrombin-antithrombin complex test (TAT) directly reflects production of intracorporeal thrombase, which increased in the early stage of DIC.

Plasmin degrades the crosslinked fibrin to release fibrin degradation products and expose the D-dimer antigen, which reflects production of intracorporeal plasmin. D-dimer will have an apparent increase with the occurrence of secondary fibrinolysis, but D-dimer test shows negative when primary hyperfibrinolysis occurs. Monitoring the above molecular markers dynamically is helpful to estimate therapeutic efficacy and guide treatment [154].

2.2.4 Diagnosis of Blood Coagulopathy

2.2.4.1 General Diagnosis

As mentioned above, clinical manifestations of blood coagulopathy in patients with severe hepatitis are lack of specificity. The most common manifestation is bleeding, not only little hemorrhage from superficial sites, but also massive hemorrhage from internal sites, such as esophagogastric varices [155]. The diagnosis of severe hepatitis complicated with blood coagulopathy is mainly based on the results of laboratory tests and clinical manifestations [156].

2.2.4.2 Diagnosis of Severe Hepatitis Complicated with DIC

According to current guidelines, basic diagnose conditions of DIC contain the following points [157].

1. Severe or multiple bleeding tendency.
2. Microcirculation collapse or shock which is difficult to explain using protopathy.

3. A wide range of embolism in skin and mucosa, focal ischemic necrosis and ulcer, or unexplained dysfunction of kidney, lung, brain.
4. Anticoagulant therapy is effective.

If severe hepatitis B patients have one of the above-mentioned points except for (1) and exhibit blood coagulating easily or prothrombin time (PT) shortening over 3 s simultaneously, it can be considered as the early stage of DIC in which the tendency of bleeding is not obvious. In addition, if severe hepatitis B patients have two of the above-mentioned four points, DIC can be considered as the preliminary clinical diagnosis. Furthermore, it can be definitely diagnosed when combined with the aforementioned items of laboratory tests (Table 2.3).

2.2.5 Prevention and Treatment of Coagulopathy

Firstly, we should use anti-viral therapy as a mainly method to treat primary disease of severe hepatitis B [158]. Then, we must eliminate the incentive, maintain the balance of water and electrolyte, and correct hypoxia and acidosis. It is very important

Table 2.3 Items of laboratory tests in DIC diagnosis

Items	Diagnosis criteria for DIC	Diagnosis criteria for DIC in severe hepatitis
① Platelet counts	<100 × 10^9/L, or with a progressive decline	<50 × 10^9/L, or with a progressive decline
② Fibrinogen (Fg)	1.5 g/L	<1.0 g/L
③ 3P test	Positive	Positive
Fibrin degradation products (FDPs)	>20 mg/L	>60 mg/L
④ Prothrombin time (PT)	Extend or shorten more than 3 s	Extend more than 5 s or changed dynamically
⑤ Plasminogen antigen (PLG:Ag)	<200 mg/L	No as a diagnostic indicator
⑥ FactorVII activity assay (FVII activity, FVII:A)	<50%	<50%
⑦ Determination of antithrombin activity III (AT-III:A)	<61%	No as a diagnostic indicator
⑧ Prothrombin fragment 1 + 2 (F1 + 2)	0.67 ± 0.19 μmol/L	0.67 ± 0.19 μmol/L
⑨ Prothrombin antithrombin complex	0.45 ± 0.4 μg/L	0.45 ± 0.4 μg/L
⑩ D-dimer (D-D)	Positive, increased more than 4 times higher	Positive, increased more than 4 times higher

Note: It can be diagnosed as DIC when there are 3 abnormalities in ① to ⑦; To test ⑧ to ⑩ items for suspected cases or early diagnosing, and at least 1 of the 3 items were positive for DIC diagnosis.

to focus on massive upper gastrointestinal hemorrhage and disseminated intravascular coagulation when treating coagulation disorders [159].

2.2.5.1 Massive Upper Gastrointestinal Hemorrhage

Gastrointestinal bleeding, including esophageal varices bleeding and non-bleeding esophageal varices, were correlated with coagulation dysfunction and portal hypertension.

Prevention

Prevention is still focused on improving the coagulation function, including adequate vitamin K1 supplements, coagulation factors, fibrinogen, fresh plasma or platelets supplements. It is particularly critical to control diet in patients with severe hepatitis. In which, light and easily digestible diet was recommended, but rough, hard and too greasy food was prohibited. For these patients, appropriate antacids can be used to protect the gastric mucosa [160].

Treatment

1. General treatment: Bed rest is necessary, meanwhile vital signs were closely monitored. The patients can eat liquid diet when bleeding mildly or having no active bleeding. However, abrosia is required when the patients have a heavy bleeding.
2. Fluid resuscitation: Firstly, intravenous access should be established rapidly in patients with gastrointestinal bleeding, Then, the patients were given intravenous infusion of normal saline, lactated Ringer's solution, plasma, whole blood or plasma substitute [161].
3. Vaso-active agents: Vaso-active agents such as dopamine and alamin may be given to maintain normal blood pressure, if blood circulation is still not stable after fluid resuscitation [162].
4. Hemostatic: If mucosal bleeding was caused by portal hypertension, oral administration of norepinephrine and ice normal saline can promote mucosal vascular contraction and hemostasis. In addition, oral administration of Yunnan baiyao may be effective [163].
5. Acid suppression therapy: The proton pump inhibitors, such as omeprazole, pantoprazole and esomeprazole, are commonly used to inhibit gastric acid secretion [164].
6. Reduction of portal pressure: Patients with severe hepatitis complicated by gastrointestinal bleeding often accompanied with portal hypertension, so it may be considered to give them drugs to decrease portal pressure. Especially in patients with portal hypertension gastropathy, reduction of portal pressure is more important than acid suppression therapy [165].
7. Compression hemostasis via using three-chamber double-balloon catheter: After the above treatment, if there is still active bleeding in patients with bleeding Esophageal Varices, it can be considered to use three-chamber double-balloon catheter [166].

8. Endoscopic hemostasis: If the above treatments have no effect in upper gastrointestinal bleeding, endoscopic hemostasis, including endoscopic hemostatic agents spray, endoscopic ligation or endoscopic injection sclerotherapy may be used [167].
9. Others: Surgery or interventional therapy may be considered, if internal medicine therapy is ineffective [168].

2.2.5.2 Disseminated Intravascular Coagulation (DIC)

Prevention

Firstly, we should treat original disease, then improve coagulative function in those patients. In addition, prevention and control of infection, correction of electrolyte disturbance, avoiding the hemorrhage and reduction of allergies and transfusion reactions should be considered [169].

Treatment

The key is early diagnosis and early treatment.

1. Anticoagulant drugs: Low molecular weight heparin is the most commonly used drug in earlier stage of DIC. It is recommended to periodic test blood routine and coagulation, and dynamically observe coagulation status during medicine therapy. Others such as dextran, anti-platelet aggregation ticlopidine, salvia injection, urokinase may be effective, but it should be support by more evidence-based medicine [156].
2. Plasma and blood products: During the process of DIC formation, the patients should be transfused with fresh plasma, each 10–20 mL/kg. When they developed the stage of secondary fibrinolysis, prothrombin complex containing coagulation factors, cryoprecipitate and platelets can be supplemented due to a large consumption of coagulation factors [170].
3. Others: such as hemodialysis, anti-fibrinolytic 6-aminocaproic acid and tranexamic acid should be supported by more evidence-based medicine.

2.3 Hepatorenal Syndrome (HRS)

Jin-Hua Hu, Zi-Jian Sun, and Xiang Xu

Hepatorenal syndrome (HRS) is a common and serious complication occurring in patients with decompensated cirrhosis and liver failure, who have overt circulatory dysfunction. The 1-year incidence of HRS in patients with ascites is about 20% [171]. HRS may predict a poor prognosis in spite of it being functional reversible [172, 173].

The clinical characteristics of HRS were first described in 1963 [174]. In 1979, HRS was defined by a group of international investigators as a progressive renal

dysfunction that occurred in severe liver diseases, with features of prerenal failure (low urine sodium concentration and hyperosmolar urine) but without any improvement following volume expansion [175]. The International Ascites Club (IAC) developed HRS definition in 1996, as a syndrome that occurs in patients with cirrhosis, portal hypertension and advanced liver failure, characterized by impaired renal function with marked abnormalities in the arterial circulation and activity of endogenous vasoactive systems [176].

2.3.1 Definition and Clinical Classification

HRS is a potentially reversible syndrome that occurs mainly in patients with liver cirrhosis, ascites and all kinds of liver failure [172]. It's clinical features include impaired renal function, marked changes in cardiovascular function and over activity of the renin–angiotensin systems and the sympathetic nervous. Progressive HRS with severe renal vasoconstriction is able to cause a decrease of GFR [177].

Clinically, HRS can be divided into two types (1 and 2). Type-1, so-called acute HRS is a rapid progressive form of renal failure defined by doubling of the initial serum creatinine concentrations to a level higher than 220 mmol/l (2.5 mg/dl) or a 50% reduction of the first 24 h creatinine clearance to <20 ml/min within 2 weeks [178]. It appears mainly in patients with acute liver failure, but often develops after a precipitating event, such as bacterial infections (especially Spontaneous Bacterial Peritonitis, SBP). The prognosis of type 1 is poor with the median survival about 1 month [177–179].

Type-2, so-called chronic HRS is a steady or slowly progressive form of renal failure defined by serum creatinine from 133 to 226 mmol/l or from 1.5 to 2.5 mg/dl [175]. Type-2 HRS is mostly related to refractory ascites. Survival of patients with type-2 HRS is generally around 6–7 months, which is better than that of patients with type-1 HRS but shorter than that of non-azotaemic cirrhotic patients with ascites. Patients with type-2 HRS tend to develop type-1 HRS while infections or other trigger events occurred [171, 177–179].

2.3.2 Pathophysiology of HRS

2.3.2.1 Portal Hypertension and Splanchnic Vasodilatation

Portal hypertension is the essential factor of haemodynamic changes, which resulted from the development of cirrhosis associated with distortion, compression and even obliteration of the hepatic sinus and vessels [175]. In patients with portal hypertension, bacterial translocation is increased and intrahepatic hypercontractile stellate cells activated [180, 181]. This overall increased resistance to portal hypertensional causes increased local production of various vasodilators such as nitric oxide, leading to splanchnic vasodilation [182, 183]. There are several other factors contributing to the splanchnic vasodilation, including hyporesponsiveness of the splanchnic

vessels and mesenteric vascular hyperplasia [181]. In addition, portal hypertension per se can cause renal vasoconstriction by activating sympathetic nervous. For example, when TIPS is used to reduce portal hypertension and improve renal blood flow, a corresponding reduction in sympathetic nervous activity has been observed [184, 185]. As a result of splanchnic vasodilation, blood is accumulated in the splanchnic vascular bed just like a splanchnic steal syndrome [186]. The combined effect leads to reduction in the effective arterial blood volume (Relative hypovolemia) causing a relative inadequacy in the systemic circulation, which triggers a hyperdynamic circulation in these patients [187, 188].

Vasodilatation induces activation of neurohumoral systems including the renin-angiotensin-aldosterone system (RASS); sympathetic nervous system (SNS); and non-osmotic release of antidiuretic hormone (ADH) [189]. Relative hypovolemia initially causes sodium and water retention, increases intravascular volume, and simultaneously increases cardiac output. As cirrhosis progresses, vasodilatation aggravates, which activated vasoconstrictive systems, causing renal vasoconstriction and decreased renal blood flow [181, 190]. Local release of potent vasodilators such as nitric oxide (NO) leads to splanchnic visceral vasodilation, as well as enables the splanchnic circulation against a variety of vasopressor agents, including norepinephrine, vasopressin, angiotensin II and endothelin [191]. The resistance of the splanchnic circulation to these vasopressor agents makes the control of arterial pressure during cirrhosis dependent on the extra-splanchnic effects produced by the endogenous vasoconstrictor systems. The role of vasoconstrictors in maintaining haemodynamic stability becomes pivotal as arterial vasodilatation increases during cirrhosis, which makes clear why cirrhotic patients with HRS are prone to develop renal, hepatic and cerebral vasoconstriction [189].

2.3.2.2 Excess Renal Vasoconstriction and Abnormal Renal Auto-regulation

The reduction of effective arterial blood volume leads to the compensatory activation of various vasoconstrictor systems. Normally, the kidneys increase the production of renal vasodilators including prostaglandins and kallikrein to maintain blood flow. However, renal vasodilator production is generally reduced in patients with cirrhosis, thus contributing to renal vasoconstriction. This type of renal hypoperfusion further increases the production of various intrarenal vasoconstrictors such as angiotensin II and endothelin, causing further decline of renal haemodynamics and renal function, occasionally accompanied by glomerular ischaemia and mesangial constriction [178].

When blood pressure fluctuates, renal auto-regulation regulatory mechanisms initiate to make sure that the kidneys receive a relatively constant blood supply. When the critical threshold is below 65 mmHg, renal blood flow is proportional to renal perfusion pressure which, in turn, is dependent on mean arterial pressure. In cirrhosis, with the development of liver disease in patients of cirrhosis, the renal auto-regulation curve gradually shifts to the right -which means as liver disease advances, the renal blood flow gradually decreases for each given renal perfusion pressure [178]. Furthermore, lumbar sympathetic blockade increases renal blood

flow in patients with HRS, suggesting that the renal sympathetic activity is involved in this outgoing hepatorenal arm.

2.3.2.3 Abnormal Cardiocirculatory Function

Insufficient cardiac output is considered one of the leading causes for renal hypoperfusion in patients with HRS in recent years. Despite control of infection, the cardiac output of cirrhotic patients with SBP who developed progressive renal failure was lower than that in similar SBP patients without renal failure. Similarly, when patients with non-azotaemic cirrhotic patients who developed HRS are compared with similar patients who did not, it is observed that low cardiac output and high plasma renin activity (PRA) were independent predictors of HRS. In addition, in patients developing HRS, the evolvement of circulatory dysfunction leading to arterial hypotension and renal failure occurs in the setting of a continued decline in cardiac output and increase in PRA. Therefore, effective hypovolaemia occurs when cardiac output decreases, resulting in renal hypoperfusion and HRS [192].

To summarize, the principal mechanisms leading to renal vasoconstriction include systemic circulation changes, accompanying portal hypertension which are characterized by decreased peripheral vascular resistance with subsequent vasodilatation, hyperkinetic circulation and the activation of compensatory mechanisms, i.e., the SNS, RAAS, and ADH. With the progression of cirrhosis, the combined effective of all the above factors will result in the gradual deterioration in renal function. Any event that leads to a sudden deterioration in hemodynamics can cause a rapid renal dysfunction, precipitating type 1 HRS [193].

2.3.3 Diagnosis

The diagnostic criteria for HRS have been first defined by IAC in 1996 [176]. The main findings include reduced glomerular filtration (creatinine clearance) less than 40 mL/min or serum creatinine increased more than 135 μmol/L after excluding the other causes of renal dysfunction. However, estimation of renal function by using creatinine clearance is not reliable, because these patients have lower levels of serum creatinine and higher renal tubular creatinine secretion compared with filtered creatinine. Furthermore, it is often incomplete for the collection of a 24 h urine.

IAC developed the new definition and diagnostic criteria for HRS in 2005, which (1) excludes creatinine clearance because of its inaccuracy of renal function estimation and the complicity to perform; (2) includes renal failure at the time of combined bacterial infection (but absence of septic shock), indicating that HRS can be diagnosed before antibiotic treatment; (3) determines by using albumin for plasma volume expansion better than saline. (4) excludes minor diagnostic criteria (urinary indices) because of its poor sensitivity and specificity for the diagnosis [178, 189, 194].

The diagnostic criteria of HRS for patients with liver cirrhosis are as follows:

1. Cirrhosis with ascites;
2. Serum creatinine >133 mmol/l (1.5 mg/dl);
3. No improvement in serum creatinine (decrease to a level of ≤133 mmol/l or 1.5 mg/dl) after at least 2 days of diuretic withdrawal and volume expansion with albumin. The recommended dose of albumin is 1 g/kg body weight/day up to a maximum of 100 g/day;
4. Absence of shock;
5. No current or recent treatment with nephrotoxic drugs;
6. Absence of parenchymal kidney disease as indicated by proteinuria >500 mg/day, microhematuria (>50 red blood cells/high power field) and/or abnormal renal ultrasonography [177, 194].

There are some other causes of renal failure in patients with cirrhosis that were not included, such as membranoproliferative glomerulonephritis and/or IgA nephropathy in patients with chronic liver diseases. These chronic forms of kidney disease can cause acute rises in serum creatinine. Determining whether it is a potential kidney disease or HRS that causes a sudden increase in serum creatinine in patients with cirrhosis and chronic kidney disease could be difficult [195].

2.3.4 Treatment

2.3.4.1 Treatment for Type-1 HRS

Liver Transplantation

Naturally, liver transplantation is the only rational solution in cases of advanced liver disease while it is also the treatment of choice for both type-1 and type-2 HRS. As calcineurin inhibitors (ciclosporin and tacrolimus) may induced GFR impairment, it is recommended to delay their administration until a partial recovery of renal function is recorded, usually 48–72 h after transplantation [177]. Clinically, the haemodynamic associated with HRS as well as neurohormonal abnormalities fade away within one month of transplantation, and the patients recover their ability to excrete sodium and free water.

Compared with patients without HRS, patients with HRS tend to have more complications, take more days in intensive care units and have higher in-hospital mortality rates after liver transplantation. Nevertheless, their 3-year survival rate is acceptable (60% vs. 70–80% in liver transplant patients without HRS [196].

The primary limitation of liver transplantation is that most patients with type-1 HRS die before transplantation due to the shortage of donor liver and their extremely short survival time. Reference to the model of end-stage liver disease (MELD, including Scr, TBIL, INR) for organ prioritisation has partially addressed this problem, since patients with HRS have a high priority on the waiting list. In addition, the

use of vasoconstrictors and albumin in the treatment of type-1 HRS can improve survival rate of these patients, and increase the likelihood of their transplantation [177].

In patients with advanced liver disease and the renal insufficiency, simultaneous liver and kidney transplant (SLKT) is taking into consideration. However the reversibility of renal function in some patients when they receive SLKT should be taken into account. Therefore, to ensure allocation of transplants only to those truly in need, the transplant community proposed an evaluation algorithm in 2006, whose purpose is to determine the presence of kidney disease with structural damage (preferably on biopsy) before giving SLKT. In the case of chronic kidney disease, a decreased creatinine clearance at 30 ml/minute or less is considered an indication of SLKT. SLKT should not be performed for the patients with simple HRS, but for the patients with HRS who become dialysis dependent and without any recovery after 6 to 8 weeks of dialysis [179, 189].

Vasoconstrictors and Albumin

Vasoconstrictors combined with albumin are the first line of therapy for type-1 HRS patients.

It was recognized long time ago that the effective plasma volume was reduced when patients of advanced liver diseases complicated with HRS, and this led to many attempts to improve the patients' renal function by expanding their plasma volume, including a large dose of albumin or saline perfusion. With the advent of safer compounds including terlipressin, a vasopressin analogue with longer activity, and the α2-agonist midodrine combined with octreotide the analogue, vasoconstrictors is widely used in the patients with HRS. These vasoconstrictors are able to ameliorate vasodilatation while increasing effective arterial blood volume, improving renal vasoconstriction and improving renal flow. In order to further increase effective blood volume, Vasoconstrictors have been used in conjunction with intravenous albumin. The clinical results from 12 uncontrolled studies including 258 patients with HRS (Type-1, 240) showed that a total of 60% were observed complete response (mostly defined as a decrease in SCr to 1.5 mg/dL). Interestingly, once the treatment is stopped, HRS relapses only in a few "responders" [177, 189].

There were several randomized controlled trials (RCTs) published, suggesting that terlipressin was associated with an increase in GFR compared with albumin alone or with a placebo. The rate of HRS reversal in the terlipressin group was higher than that in the control group (46% vs. 11%). As survival rate was not improved in the two largest RCTs, liver transplantation is still the preferred treatment for HRS, but terlipressin seems to serve as a "bridging" treatment. Two recent small, open-label RCTs suggested that the incidence of HRS reversal and the rate of side effects showed no significant difference between the two groups of norepinephrine and terlipressin [189, 197, 198].

The initial dose of terlipressin recommended in many studies ranged from 0.5 to 1 mg per 4–6 h [199, 200]. If the creatinine level did not decrease by 25% on the third day, the dose could be increased to 2 mg every 4 h or 12 mg/days by continuous intravenous infusion, respectively. In some studies, the daily dose of albumin

was generally 20–40 g by a load of 1 g/kg body weight. Some mentioned central venous pressure to establish albumin doses and to prevent body fluid from overloading. This treatment was maintained until HRS is reversed, but did not exceed 2 weeks [177].

About 20% of patients relapsed after the treatment withdrawal. However, these patients should be given repeated treatment with terlipressin, which is often effective [177].

Transjugular Intrahepatic Portosystemic Stent-Shunt (TIPS)

Several studies have evaluated the role of transjugular intrahepatic portosystemic stent-shunt (TIPS) in HRS. these studies show that TIPS help decreasing in SCr in most patients, even in a minority of organic renal failure, but it is slower compared to those obtained using terlipressin combined with albumin [201]. Recrudescence of HRS is rare provided the shunt remains patent, while hepatic encephalopathy often comes. It is worth noting that the vast majority of patients included in these studies suffered from alcoholic cirrhosis, many of whom have active alcoholism, and therefore the improvement observed may be caused by the improvement in an acute-on-chronic process. In addition, since all these studies excluded patients with a Child-Pugh score ≥ 12, resulting in a lack of data, the efficacy of TIPS should be further explored in RCTs [189].

Extracorporeal Albumin Dialysis

Molecular adsorbent recirculation system (MARS) is designed for making clearance of water-soluble cytokines (i.e., IL-6) and albumin-bound toxins (i.e., bile acids) which is implicated in the pathogenesis of HRS. Two studies showed that MARS was ineffective in improving survival rate and emic haemodynamics in type-1 HRS [193, 202]. Another clinical observation including 32 patients with type-1 HRS reported a rate of complete renal response of 28% [201]. Extracorporeal albumin dialysis (ECAD) reduces serum creatinine levels, but it is not clear whether this effect is due to a real improvement of renal function or merely a filtration process. Several studies demonstrated that patients' systemic haemodynamics improved after ECAD, manifested as an increase in systemic vascular resistances and arterial pressure, as well as a decrease in cardiac output, PRA and levels of norepinephrine. However, there were too few studies on the effect of ECAD on survival in type-1 HRS patients to draw any definitive conclusions [203, 204]. In addition, ECAD is very expensive and therefore not suitable for wide and rapid clinical application [177].

2.3.4.2 Treatment for Type-2 HRS

The treatment of type-2 HRS should take into account the survival rate as well as controlling the ascites.

Transjugular Intrahepatic Portosystemic Stent-Shunt (TIPS)

Both HRS-1 and HRS-2 are indications for the TIPS treatment. The therapeutic effect of TIPS is excellent for its better controlled of complications of portal

hypertension compared with other treatments. TIPS have been reported not only to improve renal function in patients with type-1 HRS but also to treat refractory ascites in patients with type-2 HRS [205–207].

The contraindications to the creation of TIPS are shown in the followings [195].

- **Contraindications to Placement of a TIPS:**
  - **Absolute**
    - Primary prevention of variceal bleeding
    - Congestive heart failure
    - Multiple hepatic cysts
    - Uncontrolled systemic infection or sepsis
    - Unrelieved biliary obstruction
    - Severe pulmonary hypertension
  - **Relative**
    - Hepatoma, especially if central
    - Obstruction of all hepatic veins
    - Portal vein thrombosis
    - Severe coagulopathy (INR >5)
    - Bilirubin >5 mg/dl
    - Thrombocytopenia <20,000/cm3
    - Moderate pulmonary hypertension

There were only a few studies evaluated the role of TIPS in type-2 HRS and the number of cases was quite low. In most patients, TIPS could decrease SCr, even in some with organic renal failure [208]. HRS recurrence is rare as long as the shunt remains patent, but hepatic encephalopathy often occurs [189]. Nine patients were followed-up for 1 month after the treatment of TIPS in a study, eight cases were found with decreased SCr decreased and notably controlled ascites. Four patients died, two of them died within 1 month, the other two died at 12 months and 14 months respectively. The remaining five patients survived for a long time. Although TIPS can be used in improving refractory ascites which often contributes to type-2 HRS, data on the effect of TIPS on survival are still insufficient. Therefore, the efficacy of TIPS should be further explored in randomized controlled trials (RCTs) [189].

Vasoconstrictors and Albumin

The information about combining albumin and vasoconstrictive agents treated in Type-2 HRS is limited. Only a few patients with type-2 HRS have been specifically treated with terlipressin and albumin. In one clinical study, 39 patients with HRS-2 were assigned to receive this treatment and 21 of them achieved improvement of renal function. However after the treatment withdrawal, 11 HRS patients showed relapsed during the follow-up. The most common side effects during terlipressin therapy are cardiovascular and ischemic and reported as an incidence of nearly 12%. The high recurrence rate of HRS after terlipressin and albumin treatment

discontinuation suggests that they are less effective in treating type-2 HRS compared to type-1HRS [209].

2.3.5 Prevention of HRS

Prevention of HRS is important because it develops at a constant frequency in cases of spontaneous peritonitis (SBP) and advanced liver disease [210, 211]. It becomes possible to prevent HRS if SBP is diagnosed and treated promptly [212]. According to current data, Using albumin in combination with antibiotics for the treatment of patients with SBP seems to be warranted but only for those with jaundice or renal dysfunction. The prophylactic use of antibiotics in cirrhosis with gastrointestinal bleeding also seems to be necessary, because the use of antibiotics contributes to reducing incidence of infection and rebleeding whereas improving survival rate. Furthermore, the incidence of HRS in SBP patients decreases by albumin administration, and prevention of HRS can also be related to increased survival. The recommended dose of albumin is 1.5 g/kg body weight on the first day then 1 g/kg body weight on the third day, a maximum of 150 g and 100 g, respectively. Albumin administration is strongly recommended in SBP patients with serum bilirubin levels higher than 68.4 mmol/L (4 mg/dl) or serum creatinine more than 88.4 mmol/l (1 mg/dl).

A placebo-controlled RCT that enrolled the patients with low (<1.5 g/L) ascites protein who also had advanced liver diseases or "renal dysfunction"(defined as SCr $\geq$ 1.2 mg/dL or blood urea nitrogen$\geq$25 mg/dL, or serum sodium level $\leq$ 130 mEq/L) suggested that oral norfloxacin contributed to a reduced HRS incidence within 1 year (28% vs. 41%) and an improvement in survival at the end of 3 months [213]. Norfloxacin may ameliorate or prevent vasodilatation by reducing bacterial translocation and overt infections, as well as suppressing plasma renin activity, thereby prevent these patients from developing HRS.

The concept that the severity of the clinical course of patients with cirrhosis complicated with serious bacterial infection is related to the degree of an impairment of circulatory function, which has led to new and effective approaches in the prevention and treatment of these complications.

2.4 Section 4: Fluid and Electrolyte Imbalance and Acid-Base Imbalance

Jian-Xin Song and Min-You Xin

In patients with severe hepatitis, multiple causes may lead to disorders of internal environment, mostly manifesting fluid and electrolyte imbalance as well as acid-base imbalance, usually resulting in deterioration, greater complexity and even death. Accurately recognizing the occurrence of severe hepatitis with complications such as fluid and electrolyte imbalance and/or acid-base imbalance, and therefore

giving appropriate treatment to maintain balance of internal environment, is of great importance for improving prognosis of the patients [214].

2.4.1 Physiological Basis of Fluid, Electrolyte and Acid-Base Balance

Water is ~~the~~ major component of human body. Electrolytes are substances that dissociate in solution to form charged particles, orions. Body fluid comprise mainly of water and electrolytes and electrolytes comprise mainly of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, HPO_4^{2-} and SO_4^{2-}. The primary function of electrolyte include: (1) to maintain osmotic pressure and acid-base balance of body fluids; (2) maintain nerve, muscle, cardiac cells resting potential, involved in the formation of action potentials; (3) involved in metabolism and physiological activities [215].

Body fluid include intracellular fluid and extracellular fluid, the latter can be divided into plasma and interstitial fluid. Intracellular and extracellular fluid differ in ion components. Na^+ is major cation in extracellular fluid and its main anions are Cl^- and HCO_3^-. K^+ is major cation in intracellular fluid and its major anion is HPO_4^{2-}. The total number of ions in body fluids is called osmolality, its unit is mOsm/L. If the osmolality on both sides of a semipermeable membrane is not equal, water moves toward the side with the higher osmolality. This phenomenon is called osmosis [216]. Osmosis of water can be opposed by applying a pressure across the semipermeable membrane in the direction opposite to that of the osmosis. The amount of pressure required to oppose the osmosis is defined to be osmotic pressure. In spite of various solute concentrations are different in extracellular and intracellular fluid, the osmotic pressure remain equal. Normal plasma osmotic pressure is 280–310 mOsm/L. Steady osmotic pressure is the basic guarantee to maintain the fluid balance across the cell membrane.

Multiple mechanisms in nervous and hormonal system are involved in the regulation of body fluid and electrolyte balance [217]. (1) There exists sensation of thirst in central nervous system, which plays an important role in regulating body water. (2) There is a powerful feedback system for regulating plasma osmolarity and sodium concentration that operates by altering renal excretion of water independently of the rate of solute excretion. A primary effector of this feedback is called antidiuretic hormone (ADH) which plays an important role in regulation of renal concentration and dilution to maintain the body fluid homeostasis. (3) Renin-angiotensin-aldosterone system (RAAS) is an important regulator of sodium reabsorption and potassium secretion by the renal tubules.

Human body fluid environment must be suitable pH value for maintaining normal metabolism and physiological function, Under normal conditions, human body take in acidic and basic food and drinking water, produce acids and bases during metabolism and eliminate acidic or basic substances by the kidneys and lungs. In the plasma, the normal pH value ranges from 7.35 to 7.45 with an average value at 7.40.

The regulation of the acid-base balance is accomplished by the buffer system of the body fluid, the respiration of the lungs and the excretion of the kidney [218]. (1)

Blood buffer system is composed by a weak acid and its corresponding buffer base, includes bicarbonate buffer system, phosphate buffer system, plasma protein buffer system, hemoglobin and oxygen synthetic hemoglobin buffer system. (2) The role of the lung in acid-base balance is to adjust the concentration of plasma carbonic acid by changing the amount of CO_2, so that the ratio of HCO_3^- and H_2CO_3 in the plasma is close to normal, so that the pH is relatively constant. (3) The major role of the kidneys in maintaining acid-base balance is to conserve circulating stores of bicarbonate and to excrete H^+. The kidneys maintain pH by increasing urinary excretion of H′ and conserving plasma $HCO_3^-$when the blood is too acidic, or increasing urinary excretion of HCO_3^-, and decreasing urinary excretion of H^+ when the blood is too alkaline.

2.4.2 Fluid and Electrolyte Imbalance of AECHB

2.4.2.1 Water Retention

Patients with Severe hepatitis are prone to develop water retention, with the main manifestation of seroperitoneum (ascites) as well as body weight gain. With the acatharsia of water becoming more serious, oliguria and edema of lower extremities occur. SBP (spontaneous bacterial peritonitis) can also occur, which is manifested with symptoms such as fever and abdominalgia.

Several factors contribute to ascites include an increase in capillary pressure due to portal hypertension, obstruction of venous and lymph flow through the liver, decrease in colloidal osmotic pressure due to impaired synthesis of albumin by the liver, salt and water retention by the kidney [219]. Some theories have been used to explain the increased salt and water retention by the kidney. Because of vasodilation or an actual loss of fluid into the peritoneal cavity, the effective blood volume maybe reduced, which may in turn decrease the renal blood flow leading to a lower glomerular filtration rate (GFR) and an activated rennin-angiotensin-aldosterone system (RAAS).

The diagnosis of water retention depends on typical clinical symptoms such as ascites, pleural effusion, and edema of lower extremities, when the body begins to excess water, blood pressure is increased, which leads to many complications such as congestive heart failure and pulmonary edema.

2.4.2.2 Hyponatremia

Hyponatremia is a common complication in severe hepatitis patients, and always incorporate with the retention of water and sodium, but the total sodium can be decreased, normal or even increased, that is dilutional hyponatremia. In laboratory test, serum sodium is below 135.0 mmol/L.

The mechanism of hyponatremia probably depends on the following factors: (1) the decreased function of ADH inactivation in liver brings ADH increase, enhancing the reabsorption of water in renal tubule, which causes the formation of water retention. This is the main cause of dilutional hyponatremia. (2) Water retention brings about volume extension, causing the aldosterone secretion decrease, which

leads to the sodium egestion increase in urine. (3) Some severe hepatitis patients frequently vomit, and can not eat, bringing about a major loss of body fluid and electrolyte. (4) Severe hepatitis patients, serum albumin reduces, combining with the factors such as poor appetite, anorexia, fasting or limit sodium, bring about a state of low permeability in the cell, which causes the extracellular Na + moving into the cell. (5) Iatrogenic factors, exhaust potassium diuretic such as hydrochlorothiazide and furosemide and spironolactone have a strong role in the excretion of sodium, so a large number diuretic is liable to hyponatremia in ascites patients, and in the treatment of cerebral edema, a large infusion of mannitol may cause hyponatremia either [220].

Hyponatremia due to the osmotic pressure of extracellular fluid decreased, water moves to the cells, causing cell edema, especially brain edema. So the symptoms of nerve system are the main manifestation in hyponatremia patients [221]. Generally dilutional hyponatremia in severe hepatitis patients develops slowly and progressively, and the symptoms are often covered by primary disease symptoms, like weak, feeble, nausea, vomiting, lethargy, significant body weight increase, pale and moist skin and sometimes saliva, tears increase. Improper treatment will bring about a sharp serum sodium decrease in the short term, such as serum sodium rapidly decreasing to below 125 mmol/L, acute hyponatremia syndrome comes, the manifestation include convulsions, coma, hypotension, pulse narrowing, tachycardia, oliguria even respiratory arrest and death. If cerebral hernia happens, corresponding nerve location signs will follow.

2.4.2.3 Hypokalemia

Hypokalemia refers to the condition in which the concentration of potassium (K^+) in serum is less than 3.5 mmol/L. It could occur during the whole period in severe hepatitis patients and is more common in early metaphase of disease.

Hypokalemia can be the result from one or more following medical conditions: (1) Insufficient intake of potassium due to the poor appetite or anorexia in the patients with severe hepatitis. (2) Frequent vomiting leads to excessive loss of stomach acid, which causes alkalosis and extracellular potassium is transferred into cells. (3) The reduce of effective circulating blood volume can cause to high aldosterone levels and excessive urinary losses of potassium. (4) The decreased function of aldosterone inactivation in liver brings aldosterone increase, enhancing urinary losses of potassium. (5) Some medications such as diuretics can also cause urinary losses of potassium.

The clinical syndromes of hypokalemia are related to the degree of the shortage of intra/extracellular potassium and disorders of other electrolytes and acid-base, but more depends on how soon it occurs. In the early time it shows muscle weakness, first in limbs, then develops to the torso and respiratory muscle. Deficiency of potassium also can lead to weak peristalsis, poor appetite, sick and constipation in mild hypokalemia but abdominal distention and paralytic ileus in severe situation. In cardiac syndromes, it mainly presents atrioventricular block and arrhythmia which including premature ventricular contraction or atrial premature beats, sinus bradycardia, paroxysmal auricular tachycardia or junctional tachycardia, even ventricular fibrillation. In hypokalemia state an increasing shift of potassium from

extracellular fluids into cells and an obligate loss of potassium from kidney can cause metabolic alkalosis and abnormal acidic urine. Long-term hypokalemia also can lead to hypokalemic nephropathy with proteinuria and cylindruria syndrome.

The changes of ECG [222]: In the early stage, flattened T wave and an obvious U wave, ST-segment depression can be found, QU interval is widen. In severe situation, a wide PR interval, low voltage, wide QRS interval and ventricular arrhythmia can occur.

2.4.2.4 Hyperkalemia

Hyperkalemia refers to the condition in which the concentration of potassium (K^+) in serum is higher than 5.5 mmol/L. It is more common in the middle and late period of severe hepatitis.

The mechanism of hyperkalemia: (1) The most usual way lead to hyperkalemia is oliguria or uroschesis which are caused by renal dysfunction among the patients have severe hepatitis with hepatorenal syndrome. (2) Metabolic acidosis and Na^+-K^+-ATP enzyme inactive lead to a shift of potassium out of cells also contribute to develop hyperkalemia. (3) Long-term and high dose potassium-sparing diuretics applied during the treatment lead to hyperkalemia is not rare, and easy to get sudden death in patients.

Hyperkalemia mainly influences myocardium and skeleton muscle. The most dangerous situation is fatal arrhythmia.

When the concentration of K^+ is higher than 5.5–6.5 mmol/L, there are peaked T waves. When it is over 7–8 mmol/L, PR interval is widen and P wave is flattened even vanish. When it is up to 9–10 mmol/L, T waves and QRS complex can evolve to sinusoidal shape and cardiac arrest.

Hyperkalemia is a common critical and severe symptom in clinic. When it happens, all of the potassium-sparing diuretics and potassium uptake should be stopped. At meantime, the treatment against the toxicities to myocardium and skeleton muscle should be taken to accelerate a shift into cells and potassium excreting.

2.4.2.5 Other Electrolytes Disorders

There also can happen hypocalcemia, which shows neuromuscular excitability, cardiac electrical instability and instable emotion. The concentration of calcium in serum under 2 mmol/L is significant in diagnosis.

Hypomagnesemia can be found too. It mainly presents similar symptoms as hypocalcemia such as weakness, muscle cramps, increased irritability, tetany and Chvostek positive. Hypomagnesemia can cause cardiac arrhythmia, when it occurs, the concentration of magnesium is less than 0.75 mmol/L, and it should be urgently treated.

2.4.3 Acid-Base Imbalance of AECHB

Severe hepatitis patients prone to acid-base imbalance [223], mainly to alkalemia. The main types of acid-base imbalance include respiratory alkalosis, metabolic alkalosis, respiratory alkalosis plus metabolic alkalosis, secondly include

respiratory alkalosis plus metabolic acidosis, triple acid-base disorders (TABD), metabolic alkalosis plus metabolic acidosis [224].

History and clinical manifestations provides important clues for the judgment of acid-base imbalance. The result of blood gas monitoring is the decisive basis for judging the type of acid-base imbalance. Serum electrolyte examination is an important reference.

Anion gap (AG) has important diagnostic value in determining the type of acid-base imbalance [225]. AG = Na^+-(Cl^- + HCO_3^-) is a simple formula for the value between the number of cations and anions in serum. Its normal value was 12 ± 4 mmol/L. Ag can not only help diagnose "potential" metabolic acidosis and to distinguish different types of metabolic acidosis, can also help determine special types of mixed acidosis, and has its unique role in the judgment of TABD.

Sometimes the indicators of blood gas analysis are normal, the calculation of AG value become the only evidence of diagnosis of metabolic acidosis. In addition, AG value can be used as reference for correction of acid-base imbalance. In severe hepatitis, the change of AG values can be used as an indicator to estimate the complications and prognosis. Clinical observations indicate: AG value significantly increased often suggestive of severe infection, kidney dysfunction or severe bleeding, and the prognosis is poor.

2.4.3.1 Respiratory Alkalosis

Respiratory alkalosis refers to arterial $PaCO_2$ decrease and pH > 7.45 as well as compensatory decrease of blood HCO_3^-. Respiratory alkalosis occurred in early stage of severe hepatitis.

In severe hepatitis, respiratory alkalosis related to hyperventilation: accumulated ammonia and other vasoactive peptides excited respiratory center, ascites and pleural fluid increase respiratory rate, hypoxemia excited respiratory center.

Compensatory mechanisms: CO_2 reduction, breathing shallow and slow, so that CO_2 retention, H_2CO_3 compensatory rise; when last longer, reduce renal row H^+, HCO_3^- excretion increased, HCO_3^-/H_2CO_3 equilibrium at a low level.

Most patients have the performance of shortness of breath and heart rate increase. Can have vertigo, hand, foot and mouth numbness, muscle tremor, hand and foot convulsions. Convulsions associated with low calcium. Dysfunction of nervous system is related to the damage of brain function and cerebral blood flow decrease.

Respiratory alkalosis diagnosis relies on the following: (1) pH is normal when fully compensated, increased underdecompensation. (2) $PaCO_2$ lower (typically <35 mmHg or 4.67kpa). (3) HCO_3^- compensatory decline. (4) AG value may have a slight increase. (5) blood Cl^- may increase.

2.4.3.2 Metabolic Alkalosis

Metabolic alkalosis refers to the type of acid-base imbalance characterized by an increase HCO_3^-in extracellular liquid.

The inappropriate application of basic drugs, potassium-sparing diuretics, dehydrating agents, hormones can often induce or aggravate metabolic alkalosis. Severe

gastrointestinal symptoms, anorexia, vomiting or diarrhea are also the reasons for the occurrence of metabolic alkalosis.

Compensatory mechanisms: when alkaline substances increased in body, buffer system instantly transfer strong base into weak base, increase HCO_3^- consumption, H_2CO_3 increased. Inhibit respiratory center, decrease pulmonary ventilation, CO_2 retention, HCO_3^- compensatory increase. Renal carbonic anhydrase activity decreased and H^+ formation and excretion decreased, $NaHCO_3$ reabsorption is also reduced, so HCO_3^-/H_2CO_3 compensatory restore to 20:1, pH value is normal.

Patients with mild metabolic alkalosis usually have no obvious symptoms. Many disorders can occur in severe metabolic alkalosis. (1) Functional changes in the central nervous system, the patient may have irritability, confusion, delirium, consciousness disorders. (2) Slow and shallow breathing, hypoxemia. Brain tissue is particularly sensitive to hypoxia, thus neurological symptoms first appeared. (3) Hypocalcemia and neuromuscular stress increased, the performance of tendon hyperreflexia, face and muscle twitching, and limbs twitching. (4) Hypokalemia can cause neuromuscular symptoms and arrhythmias.

According to pH value, $PaCO_2$, HCO_3^-, level of K^+ and Cl^-, effective circulating blood volume and performance of primary disease, diagnosis of metabolic alkalosis is no difficult to make. Metabolic alkalosis should be divided into two categories based on the urinary level of Cl^-. (1) chloride positive metabolic alkalosis: supplement sodium chloride can correct the alkalosis. It indicates that the body has Cl^-deficiency, urinary $Cl^- < 10$ mmol/L. (2) chlorine negative metabolic alkalosis: alkalosis can not be corrected by supplement sodium chloride, urinary $Cl^- > 20$ mmol/L.

2.4.3.3 Respiratory Alkalosis Plus Metabolic Alkalosis

Respiratory alkalosis plus metabolic alkalosis tend to occur on the early stage of severe hepatitis. In most cases, there is no obvious complication, more often metabolic alkalosis happens on the basis of respiratory alkalosis, or the other way around.

Due to respiratory and metabolic factors are inclined to alkaline change, name as reduce $PaCO_2$ and elevated plasma HCO_3^-, there is no mutual compensation between them, so it is easy to present as severe decompensation and poor prognosis. Main point of diagnosis: (1) pH value of blood increase significantly. (2) $PaCO_2$ decrease. (3) HCO_3^- increases, the value should be greater than $0.5\times(40\text{-}PaCO_2) + 2.5$. (4) Hypokalemia and hypochloremia are common phenomenon.

2.4.3.4 Respiratory Alkalosis Plus Metabolic Acidosis

Respiratory alkalosis plus metabolic acidosis is relatively rare. Metabolic acidosis can be divided into 3 types: (1) value of AG is normal (high chlorine acidosis), commonly seen at long-term diarrhea, combined with renal tubular acidosis and a large amount of physiological saline input in patients or water intoxication. (2) high AG value type (normal blood chlorine acidosis), regularly present in combination of hepatorenal syndrome, lactic acidosis and patients with ketoacidosis. (3) a hybrid type (high AG merged high blood chlorine), mainly in patients with severe diarrhea following lactic acid or ketoacidosis.

When respiratory alkalosis plus metabolic acidosis happens, $PaCO_2$ and plasma concentration of $HCO_3^-$are higher than scope of compensation to each other. Its characteristics as follows: (1) the range of blood pH change is not large, normal, slightly higher or slightly lower. (2) $PaCO_2$ reduce to less than $1.5 \times HCO_3^- + 6$ or $HCO_3^- < 24\text{-}(40\text{-}PaCO_2) \times 0.5\text{–}2.5$. (3) AG values can be normal or elevated, the latter is more common. If the elevated blood Cl^-value is equal to the HCO_3^- decrease, AG value normal metabolic acidosis type can be diagnosed; the cases that the rising value of AG is equal to the decline of HCO_3^- values can be diagnosis as AG increased metabolic acidosis type. On the third occasion, the increase value of AG is equal to the sum of HCO_3^- and Cl^- drop, the diagnosis is mixed metabolic acidosis. For this type of offset mixed acid-base balance disorders, treatment should be moderate, the measure of the metabolic factors correcting should be precede to respiratory factors, avoid $PaCO_2$ quickly returning to normal in the process of treatment, which would lead to blood pH drop rapidly and acidosis more worse. To patients with severe hepatitis, the harm of alkalosis is greater than acidosis, thus the blood pH should be kept slight acidic in a normal state. Generally, the target of alkali supplement can be arterial blood pH value recovered to 7.20.

2.4.3.5 Respiratory Alkalosis TABD

Respiratory alkalosis TABD refers to respiratory alkalosis, metabolic acidosis and metabolic alkalosis three primary imbalances coexist in the same patient, which is one sort of serious acid-base imbalance, mostly develops in the late stages of severe hepatitis, fatality rate is high.

Respiratory alkalosis TABD characteristics as follows: (1) blood pH value depends on the relative severity of these three primary imbalances, which can be normal, or slightly high generally. (2) reduce $PaCO_2$, its value is less than $1.5xHCO_3^- + 6$. (3) HCO_3^- can raise, normal or lower. (4) value of AG rise significant, and extent of AG raise is greater than the HCO_3^- lower. (5) Cl^- often lower than normal.

2.4.3.6 Metabolic Alkalosis Plus Metabolic Acidosis

The occurrence of metabolic alkalosis plus metabolic acidosis in patients with severe hepatitis is not uncommon. Usually it is accompanied with existing lactic acidosis or ketoacidosis, and the patient may manifest frequent vomiting.

Since the causes for raising and lowering HCO_3^- coexist, they tend to cancel one another. The pH and HCO_3^- concentration can be normal, increased or decreased, depending on the relative severity of the two kinds of imbalances.

2.4.3.7 Other Types of Acid-Base Imbalance

Severe hepatitis can also be accompanied by metabolic acidosis, metabolic acidosis plus respiratory acidosis, TABD of respiratory acidosis, and etc.

Pure metabolic acidosis refers to arterial blood pH < 7.35 and compensatory decline of $PaCO_2$ due to primary decrease of HCO_3^-. Typical manifestation is known as Kussmaul breathing, characterized by deeper and faster breathing, as well as

obvious contraction of respiratory muscle, and also ketone-smelled exhaled breath. The patients often flush, companied by increased heart rate and decreased blood pressure. There may be reduced or disappeared tendon reflexes, confusion or stupor.

Due to respiratory and metabolic factors both towards to acidic changes, there is no respiratory compensation for decrease in HCO_3^-, nor renal compensation for increase in $PaCO_2$, hence presenting severe decompensated status. The resulting distinct decrease in pH and vicious circle are the characteristics for metabolic acidosis plus respiratory acidosis.

The characteristics for TABD of respiratory acidosis include significantly increased $PaCO_2$, elevated HCO_3^-, AG > 16 mmol/L, and significantly decreased Cl^-.

The incidence of the above types of acid-base imbalance is very low in patients with severe hepatitis. Once it happens, it should be actively treated with corresponding methods, so that the blood pH quickly restores to the safety range. During therapeutic process against various pure acid-base imbalance, interactions among various treatments need to be taken into consideration, in order to avoiding the possibility that the treatment for one type of acid-base imbalance causes or aggravates another type.

In summary, water-electrolyte imbalance and acid-base imbalance have a relatively high morbidity in patients at various stages of severe hepatitis. This often results in deterioration and complication of the disease, evermore, the death of the patient. Therefore, the functions of heart, lung, kidney, blood circulation as well as changes of body weight in the patient need to be intently monitored. Regular detection of K^+, Na^+, Cl^-, carbon dioxide combining power (CO_2CP), blood urea nitrogen, creatinine, pH, data about arterial blood gas analysis, and also detailed records of patient's input and output are demanded. During the process of diagnosis and treatment, careful analysis of the history, clinical manifestations and laboratory examination are necessary to achieve correct diagnosis, early prevention and prompt treatment.

2.4.4 Prevention and Treatment of Acid-Base and Electrolyte Imbalance

2.4.4.1 Prevention and Treatment for Ascites

In normal condition, proper amount fluids can make a lubrication action on organs in peritoneal cavity. But in those patients who have severe hepatitis, especially with cirrhotic portal hypertension, too many fluids over 200 mL can lead to ascites. The ascites can be categorized into uncomplicated ascites and refractory ascites. There is no infection in uncomplicated ascites and won't lead to hepatorenal syndrome, but refractory ascites is in the contrast. The refractory ascites includes diuretic-resistant and diuretic-intractable ascites. The diuretic-resistant ascites shows no response to diuretic and diuretic-intractable ascites limits the application of diuretic due to the complications induced by diuretic.

Prevention and Treatment for Uncomplicated Ascites

100 mg antisterone per day as an initial dose can be given to those patients with moderate ascites, if it goes to no satisfied effect, 100 mg can be added after every 7 days till the maximum dose to 400 mg/d. If the patients show a hyperkalemia or aldosterone antagonist-resistant, nicorol can be combined and with an increasing dose from 40 to 160 mg/d gradually. The patients without edema losing weight should be less than 0.5 kg and those with edema should be less than 1 kg per day to avoid electrolyte disturbance or hepatorenal syndrome during the whole treatment period. Diuretics should be withdrawn on the patients with severe hepatic encephalopathy, severe hyponatremia, progressive renal failure or severe muscle spasm. The patients should only take the minimum dose of diuretics to maintain the state after the syndrome controlled, or withdraw when it is necessary.

Prevention and Treatment for Refractory Ascites

Due to the poor outcome and living quality, the median survival time of refractory patient is half year. So liver transplantation can be considered in the patients with refractory ascites induced by cirrhosis, which required more cautious before make a diagnosis. Generally, if the patients meet the following conditions when they are receiving 400 mg/days antisterone and 160 mg/days nicorol treatment over 1 week and sodium uptake limited in 90 mmol/days, refractory ascites can be diagnosed. (1) losing weight less than 0.8 kg/days over 4 days (2) sodium uptake is more than elimination (3) grade 2–3 ascites is arisen again after 4-week long treatment (4) hepatic encephalopathy, hepatorenal syndrome and severe electrolyte disorder induced by diuretics are shown up.

Refractory ascites patients without complications can be treated with abdominocentesis, but it will possibly induce circulation failure, and increases the risk of hepatic coma and hemorrhage. So low rate infusion of albumin with abdominocentesis is combined to avoid circulation failure, meantime diuretics also need to give after abdominocentesis. Aldosterone antagonist combined with nicorol is a suitable strategy: the dosage of antisterone is increased from 100 to 400 mg/days and nicorol from 40 to 160 mg/days gradually. The goal of this strategy is to maintain the situation without ascites under the minimum dosage. But when severe hepatic encephalopathy and electrolyte disorder show up, which means serum sodium concentration is less than 120 mmol/L, serum potassium concentration is less than 3.0 mmol/L, nicorol should be withdrawn, if serum potassium concentration is more than 6.0 mmol/L, antisterone should be withdrawn.

To those patients who need abdominocentesis repeatedly, transjugular intrahepatic portosystemic shunt (TIPS) can be considered, but the risk of hepatic encephalopathy will be higher and the outcome is poor. So the patients with severe liver and renal failure, cardiorespiratory function failure or active infection should be in cautious.

The mean survival time of refractory ascites patients complicated with hepatorenal syndrome is 3 months, preventive antibiotics combined with albumin is an option for these patients. To those patients who have already showed hepatorenal syndrome, terlipressin combined with albumin could be useful. Meantime,

abdominocentesis, TIPS or artificial liver supporting treatment can improve patients' living quality in short term, but long-term outcome won't be good, so liver transplantation should be execute as soon as possible. In general, medicine can improve the symptoms in short term but with poor outcome, liver transplantation is more meaningful.

2.4.4.2 Prevention and Treatment for Hyponatremia

Patients with hypovolemic hyponatremia can have a supplement with sodium and decrease the dosage of diuretics, patients with hypervolemic hyponatremia can restrict fluids uptake (less than 1000 ml/d) and combined with vasopressin V2 receptor blocker or antidiuretic hormone receptor blocker. Currently, vaptans, tolvaptans, conivaptan and satavaptan have already applied in clinical practice. There was research showed that vaptans could improve 45–82% patients' symptoms significantly after patients took it for 1 week to 1 month, and main side reaction was thirsty. The patients complicated with hepatic encephalopathy should be used vaptans with cautious due to its high risk in dehydration and hypernatremia. Meantime, vaptans is metabolized by CYP4A, so rifampin, barbital and phenytoin can decrease its effect, and ketoconazole, clarithromycin can increase its plasma concentration. Tolvaptans can give some relief but increase the risk of hemorrhage. Satavaptan will decrease patients' survival rate. So the proper treat period and side reactions of these drugs in long-term using need to make clear.

2.4.4.3 Prevention and Treatment for Hyperkalemia

Potassium uptake should be withdrawn immediately after hyperkalemia occurs, emergency treatment for detoxicating potassium should be taken to protect cardiac. The treatment depends on the plasma concentration of potassium.

The treatment for patients with fulminant hepatitis B with cirrhosis complicated with hyperkalemia is to restrict the uptake of potassium, improve the microcirculation, correct renal filtration decrease induced by hepatorenal syndrome and increase the elimination of potassium. There are also some patients have abnormal distribution of potassium due to hypoxia, acidosis, catabolism, and deficiency of energy supplies, which leads to intracellular potassium is transferred into extracellular. The treatment for these patients is to correct hypoxia and acidosis, high glucose, insulin and ATP are administered to boost glycogen synthesis to transfer potassium from extracellular to intracellular. Peritoneal dialysis and plasmapheresis can be given to the patients with intractable hyperkalemia.

2.4.4.4 Prevention and Treatment for Hypokalemia

Hypokalemia can present during the whole period of fulminant hepatitis B, it will occur more often on the early and middle stage. Long-term inappetency and abdominal distension lead to insufficient potassium uptake. Nausea, vomiting and diarrhea lead to increase of potassium losing. Renal filtration rate decreasing and aldosterone increasing lead to potassium elimination increase. Complicated with alkalosis and anabolism increase also can cause hypokalemia. The treatment for hypokalemia

should focus on comprehensive therapy, correcting alkalosis, increasing potassium supply, improving microcirculation.

2.4.4.5 Prevention and Treatment for Disturbance of Acid-Base Balance

Disturbance of acid-base balance occurs quit often in hepatitis B patients, especially with alkalosis. During the early stage, it can present in pure respiratory alkalosis, also can complicated with metabolic alkalosis. During the middle and late stage, both of above symptoms and metabolic acidosis occurs concurrently. Treating idiopathy and correcting hyperventilation is a treatment for respiratory alkalosis. Arginine hydrochloride injection is used to treat metabolic alkalosis to avoid secondary metabolic alkalosis. The general regulation is prefer acid to base, till pH value of arterial blood back to 7.2.

In prevention of disturbance of acid-base balance, the effective strategy is to correct hypokalemia and hypochloremia, control vomiting. Meantime, controlling infection, endotoxemia and upper gastrointestinal hemorrhage are necessary.

2.5 Hepatic Encephalopathy

Qiong-Fang Zhang and Da-Zhi Zhang

Hepatic encephalopathy (HE) due to metabolic disturbance is a complex neuropsychiatric syndrome caused by severe liver dysfunction or disorder and is one of the common complications and causes of death in severe liver diseases. Patients with HE mainly present with neuronal or mental abnormalities and disturbance of consciousness, even coma and death. The clinical manifestations and the severity of the disease vary because of its complex pathogenesis.

2.5.1 The Concept and Clinical Classification of Hepatic Encephalopathy

2.5.1.1 The Concept

Hepatic encephalopathy is the result of acute and chronic hepatic failure caused by cirrhosis or various kinds of portosystemic shunt (PSS) created. A diagnosis of HE can be made after excluding encephalon diseases. The syndrome is caused by metabolic disorders and is potentially reversible. HE clinical features differ due to the wide degree and range of neuropsychiatric symptoms that vary from subtle abnormalities detected only by intelligence tests or electrophysiological methods geared for detecting personality changes to abnormal behavior, intellectual impairment, and even different degrees of consciousness disorders. HE was previously known as hepatic coma, but that is only one of the worst severe signs of HE and does not represent all types of HE.

2.5.1.2 HE Classifications

In 2003, the World Congress of Gastroenterology (WCOG) suggested that based on the cause HE can be divided into three types (A, B, and C) [226, 227].

Type A: Type A is acute liver failure-related HE and the symptoms occur within 2 weeks. In subacute liver failure-related HE, the symptoms of HE occur within 2–12 weeks with or without predisposing factors.

Type B: Patients with type B HE have obvious PSS and normal liver histology without associated intrinsic liver disease. These clinical manifestations are similar to those in patients with HE and cirrhosis. The PSS may be spontaneous or caused by surgical or interventional procedures [228]. Common causes of PSS include congenital vascular malformation, intrahepatic or extrahepatic portal vein obstruction (including trauma, carcinoid, and bone marrow hyperplastic disease caused by a high coagulation state due to portal vein branch embolization and thrombosis) and generation of portal hypertension by oppression of lymphoma, metastatic tumors, and bile duct carcinoma.

Type C: Type C HE is related to chronic liver diseases, with cirrhosis being the most common type, and is generally accompanied by portal hypertension and PSS. Type C HE is mainly caused by liver function failure, rather than by PSS. According to the clinical manifestations, duration and characteristics, type C can be divided into three types: episodic HE, persistent HE, and minimal HE [226].

Episodic HE

Episodic HE, related to chronic hepatic disease, is defined as a disturbance of consciousness and cognitive change in a short time and can be alleviated by spontaneous remission or drug treatment in the short term, which cannot be explained by a relevant preexisting mental disorder. Episodic HE can be divided into three types according to the presence of known risk factors: (1) incentive type: there is a clear history of predisposing factors; (2) spontaneous type: there is no history of predisposing factors. (3) Recurrent type: HE attacks more than two times within a year.

Persistent HE

Persistent HE related to chronic hepatic disease is defined as an occurrence of continuous neural mental abnormality, including cognitive decline, disturbance of consciousness, coma and even death. Persistent HE can be further divided into three types according to the severity of the disturbance in the patient's self-control and self-discipline: 1, mildest type, namely West Haven level 1; 2, severe type: namely West Haven level 2–4; and 3, therapeutic resistance type: medication can alleviate HE quickly, but withdrawal can aggravate HE rapidly.

Minimal HE

Patients with minimal HE, with normal clinical manifestations and routine biochemical tests, have mild cognitive and psychomotor deficits detected by neuropsychology and neural physiology tests, and these patients usually have a history of chronic hepatic disease [229].

The prevalence of minimal HE in patients with cirrhosis is 30–80%. Patients with minimal HE with reduced physical and mental ability have gained more and more attention recently because they have a high risk of accidents when engaged in occupations involving mechanical, or driving work.

2.5.2 Pathogenesis

The pathogenesis of HE has not been fully elucidated so far, and many theories have been put forward. It is generally believed that HE is caused by acute and chronic liver failure and/or PSS. When toxic substances absorbed by the intestines cannot be detoxified and cleared by (or through) the liver, they directly enter into the systemic circulation and pass through the blood-brain barrier to reach the brain tissue and cause central nervous system dysfunction. A variety of the risk factors mentioned above can result in HE. Hyperammonemia is still recognized as one of the most important factors, especially in HE related to chronic liver disease, liver cirrhosis and/or PSS. According to the ammonia intoxication theory several factors including false neurotransmitters, such as γ-aminobutyric acid/benzodiazepine (GABA/Bz) receptor complex, an imbalance in the ratio of branched chain amino acids to aromatic amino acids, brain cell edema, astrocyte dysfunction, mercaptan, short chain fatty acid toxicity and manganese deposition are all involved in the occurrence of HE [230].

2.5.2.1 Ammonia Intoxication Theory

Ammonia intoxication caused by an ammonia metabolism disorder is the most important factor in the pathogenesis of HE [231]. Ammonia comes mainly from the gut and the generation and absorption of ammonia increase in a serious liver disease when excess ammonia cannot be cleared sufficiently by ornithine cycle due to serious damage to liver parenchyma. When PSS occurs, intestinal ammonia directly enters the systemic circulation without liver detoxification, resulting in increased blood ammonia. High levels of blood ammonia can enter the brain through the blood-brain barrier and generate central nervous system toxicity by interfering with cerebral energy metabolism, neurotransmitter and nerve cell membrane ion transport; increasing cerebral edema; and changing gene expression (such as stellate cell glutamate carrier, stellate cell structural protein, glial fibrillary acidic protein, peripheral benzodiazepine receptor and aquaporin-4) and inducing the mitochondrial permeability transition (MPT).

The main way of removing ammonia from the brain is through urea cycle. During glutamine synthesis, glutamic acid is formed from ammonia and α-ketoglutaric acid and the glutamic acid combines with ammonia to generate glutamine. This process requires ATP and consumes a large amount of α-ketoglutaric acid, which interferes with the brain energy metabolism and causes an energy supply shortage in brain cells. Glutamate is an important excitatory neurotransmitter in the brain, and lack of glutamate increases inhibition in the brain. Glutamine synthetase is present in astrocytes, where glutamic acid is detoxified to glutamine. Glutamine is a strong intracellular osmotic agent, and increases in glutamine can lead to brain cell swelling.

Reports have identified a strong correlation between the content of glutamine in cerebrospinal fluid (CSF) and the degree of HE [232]. During HE, excess ammonia under the effect of glutamine synthetase, not only reduces the formation of active glutamate but also consumes a lot of energy, leading to the accumulation of glutamine, which increases intracellular osmotic pressure and causes brain cell swelling. Swollen astrocytes with impaired function further affect ammonia metabolism, reduce the ability of neurons to efficiently uptake or release extracellular ions and neurotransmitters, and stimulate glial cell synthesis of neurosteroids by upregulating their expression of the peripheral-type Bz receptor (translocator protein, 18 kDa). Neurosteroid is an endogenous Bz that can enhance GABA nerve tension and cause symptoms in patients with HE [233] (Fig. 2.2). Recent studies have

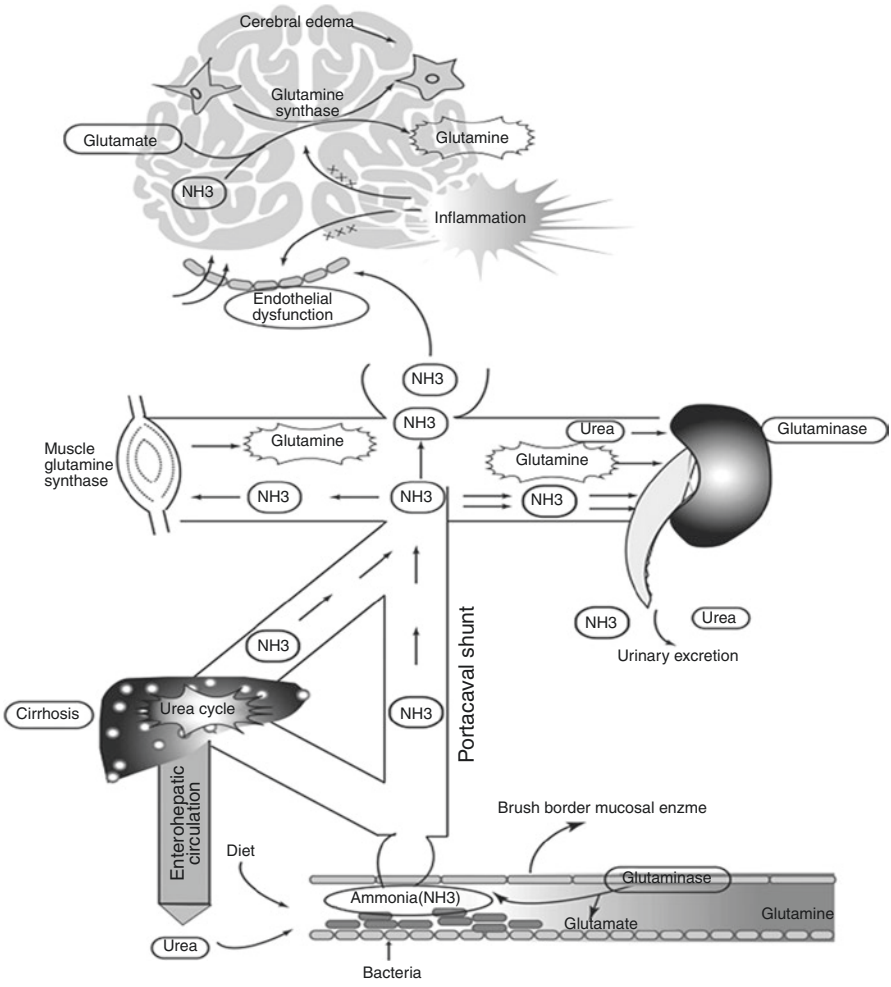

Fig. 2.2 Pathogenesis of hepatic encephalopathy

shown that the metabolic rate of cerebral ammonia in HE patients is increased. Increased levels of blood ammonia enter the brain through the blood-brain barrier. Brain dysfunction also occurs even if blood ammonia levels appear normal; this partially explains the occurrence of HE in the case of normal blood ammonia and invalidates HE treatment by simply reducing blood ammonia. In addition, increasing evidence suggests a synergistic effect between blood ammonia and its metabolic disorders with systemic inflammation, nerve steroids, oxidative stress, nitrification stress, manganese poisoning, and GABA/Bz [233].

2.5.2.2 The GABA/Bz Receptor Theory

The main inhibitory neurotransmitter in the mammalian brain is GABA. Plasma GABA is derived from the conversion of glutamic acid by glutamate decarboxylase in intestinal bacterial. Notably, GABA has dual role. On one hand, during liver function failure and PSS, the removal of GABA in liver is significantly decreased; on the other hand, GABA can directly enter the systemic circulation bypassing the liver, resulting in increased concentration of GABA in blood. The concentration of GABA in CSF and brain tissue increases as more GABA crosses the abnormal blood-brain barrier. In addition, endogenous Bz was found in the blood and CSF, and the GABA receptor on the membrane surface of the brain's postsynaptic neurons increased significantly in some patients with HE and in animal models. This receptor not only combines with GABA but also binds to barbiturates (BARB) and Bz on different parts of the receptor surface; thus, it has been named the GABA/Bz complex receptor or the super receptor complex. When liver function is severely impaired, the binding affinity of this complex receptor to its three ligands is also increased. Binding of GABA, BARB, or Bz with the complex receptor can promote entry of chloride ions from neuronal membrane ion channels into the cytoplasm of postsynaptic neurons, causing membrane hyperpolarization and nerve conduction inhibition. HE symptoms were relieved in about 30% of patients treated with a GABA receptor antagonist or Bz receptor antagonist, and GABA/Bz and ammonia were reported to act synergistically in HE. Recently, some studies focused on peripheral type Bz receptors, which are different from central GABA [234, 235]. Some questions, including the source of endogenous Bz, and the correlation between the increased degree of GABA or Bz and the disease, remain to be answered. Therefore, therapy targeted at reducing the blood ammonia concentration in patients with HE and significantly reducing the increased GABA nerve tension seems reasonable [236], but may not be completely effective. Treatment effects of reducing ammonia vary, because of the different levels of ammonia in HE patients that can be produced by the interaction between various known or unknown factors and the different effects of Bz receptor antagonists.

2.5.2.3 False Neurotransmitters and Imbalance of Amino Acid Metabolism Theory

This theory is related to the metabolism of aromatic amino acids (AAA), the precursors of true neurotransmitters, including norepinephrine and dopamine. Due to the reduction in the liver's detoxification function or formation of PSS, the amines

(phenylethylamine and tyramine) produced in the intestine cannot be cleared completely, resulting in elevated concentrations of these amines in the systemic circulation and increased levels in the brain through the blood-brain barrier. Under the effect of β-hydroxylase, phenethanolamine and β-hydroxytyramine (β-dopamine) are generated from phenylethylamine and tyramine, respectively and are similar to norepinephrine and dopamine in chemical structure. These amines can be taken up, stored and released by adrenergic neurons in the brainstem reticular structure. Phenethanolamine and β-hydroxytyramine are called false neurotransmitters because of their low physiological effects on the postsynaptic membrane, which is about 1/10 of norepinephrine. When these false neurotransmitters accumulate in the nerve synapse, they can outcompete or replace normal neurotransmitters, resulting in a disorder of nerve conduction. It was reported that plasma AAA (such as phenylalanine, tyrosine, and tryptophan) increased and branched-chain amino acids (BCAA, such as valine, leucine, isoleucine) decreased in patients with decompensated liver cirrhosis, leading to an imbalance of amino acid metabolism. AAA are decomposed and metabolized in the liver, and liver failure decreases AAA decomposition resulting in an elevated concentration of AAA in the plasma. Insulin can promote BCAAs entering muscle, which is then broken down and metabolized in the skeletal muscle instead of the liver. Insulin inactivation is decreased in patients with liver failure, promoting a large number of BCAAs entering the muscle tissue and decreasing the concentration of BCAAs in plasma. Finally, the BCAA/AAA ratio is reduced from a normal 3–3.5:1 to 1:1 or lower. The above process reduces the BCAA concentration, but increases the AAA concentration, leading to an increase in synthesis of false neurotransmitters and reduction of the normal neurotransmitter [237–239].

2.5.2.4 Manganese Poisoning Theory

The epidemiological data suggests that manganese poisoning and HE extrapyramidal have common clinical symptoms. The liver is an important organ for manganese excretion. The concentration of blood manganese can be increased when liver function is affected, during PSS, or when excretion of bile is reduced. Manganese content in plasma was sharply increased in more than 80% of patients with acute hepatitis and liver cirrhosis and the density of globus pallidus increased in the brain basal ganglia of HE patients (partially 2–7 times higher by MRI). Based on histological results, the above changes were caused by manganese deposition, which disappears after liver transplantation. It has been suggested that manganese deposition may cause dopamine dysfunction. Deposition of manganese not only cause direct brain injury, it can influence the function of 5-hydroxytryptamine (5-HT), norepinephrine and GABA neurotransmitters; impair astrocyte function; and have a synergistic effect with ammonia. However, there is no reliable correlation between the concentration of serum manganese and HE severity, which may be due to the chronic deposition of manganese [240]. The characteristic change in MRI imaging as the deposition of manganese remains to be verified. The effectiveness of manganese removal to improve the symptoms and neurological signs of patients with HE needs further validation.

2.5.2.5 Additional Theories

The synergistic toxic effects between toxins (ammonia and mercaptan) and short chain fatty acids [241], the 5-HT hypothesis, the effect of Helicobacter pylori urease, opioids, endotoxin, tumor necrosis factor, melatonin, and hepatitis B virus termed additional theories of HE syndrome. This theory also suggests the same hypothesis mentioned in the above theories.

2.5.3 Common Predisposing Factors of HE

Due to the extensive amount of liver cell damage caused by acute liver failure in type A HE, the residual liver cells cannot effectively remove toxins leading to central nervous system dysfunction. Type A HE, known as non-ammonia encephalopathy, is endogenous HE without clear causative agents. Simple type B HE is rare in mainland china; the liver can clear limited metabolic toxins in patients with chronic liver failure or PSS, but once these toxins exceed the compensatory capacity of the liver, type C HE occurs. The occurrence of type C HE is largely related to the following risk factors, which are the most important factors in the prevention and treatment of HE.

2.5.3.1 Excessive Intake of Nitrogen

Patients with chronic liver failure or PSS are less tolerant to the protein found in food, especially animal protein. A large amount of ammonia and AAA are produced by the decomposition of intestinal bacteria, which can induce HE. Oral ammonium salts, urea, and methionine can induce HE by increasing the absorption of nitrogenous substances and elevating blood ammonia.

2.5.3.2 Massive Hemorrhage in the Digestive Tract

Intestinal production of ammonia can be increased by hemorrhage in the intestine (100 ml of blood contains 15–20 g protein). At the same time, because of the lack of isoleucine in the blood, after digestion and absorption of a hemorrhage, extra blood leucine and valine increase BCAA decomposition by enhancing the activity of BCAA dehydrogenase, thereby exacerbating the imbalance in the BCAA/AAA ratio. Loss of blood volume, cerebral ischemia and hypoxia also increase the sensitivity of the central nervous system to ammonia and other toxic substances [230].

2.5.3.3 Infection and Sepsis/Systemic Inflammatory Response Syndrome, SIRS

Infections such as spontaneous peritonitis, pneumonia, and urinary tract infection can increase tissue decomposition and production of ammonia. Secondary sepsis or SIRS induce HE through TNF-α, IL-1, IL-6 and other inflammatory factors, exacerbates oxidative stress, and increases the blood-brain barrier permeability of ammonia and other toxic molecules to liver and brain [242]. Studies have shown that SIRS is directly related to the deterioration of HE in patients with liver cirrhosis, and its extent and mortality increase with the deterioration of SIRS [243]. Similarly, SIRS

is a common factor in triggering chronic liver failure characterized by HE and renal failure. In a study of patients with liver cirrhosis, artificially-induced hyperammonemia by oral administration of glutamine may have worsened the results of psycho-mental testing in 10 cases of sepsis patients; while brain toxicity was not obvious after the inflammation was relieved, the observation of decreased cytokine levels indicated that infection and induced inflammatory mediators enhanced brain toxicity of hyperammonemia. Accordingly, some researchers suggested that SIRS could be an independent pathogenesis of HE rather than a risk factor [243].

2.5.3.4 Water and Electrolyte Disturbance

Hyponatremia can affect the intracellular osmotic pressure and lead to brain edema, which induces HE. Hypokalemia is often associated with metabolic alkalosis [244]. Mass use of diuretics or extraction of ascites can also cause alkalosis. Ammonia is easily absorbed by the intestinal tract or through the blood-brain barrier inducing HE [245].

2.5.3.5 Azotemia

A variety of reasons can cause pre-renal azotemia such as hypovolemia, anorexia, diarrhea, limiting the amount of liquid, mass use of diuretics, or extraction of ascites. Hepatorenal syndrome or other causes can result in renal azotemia. Pre-renal azotemia and renal azotemia caused by hepatorenal syndrome or other causes can increase the concentration of ammonia in the blood.

2.5.3.6 Other Theories

Several other predisposing factors can contribute to HE such as constipation, hypoglycemia, the use of sedatives and proton pump inhibitors, and epilepsy. After the occurrence of constipation and intestinal obstruction, the patient's intestinal mucosa is exposed to ammonia longer thus increasing the absorption of ammonia. Hypoglycemia can reduce brain deamination. The binding of sedatives, hypnotics and the brain GABA/Bz receptor produce an inhibitory effect on the brain. It was reported that proton pump inhibitors increase the risk of HE in patients with cirrhosis in a population study [246]. Another study also suggested that epilepsy was associated with an increased risk of HE in patients with cirrhosis [247].

2.5.4 Pathological Changes in HE

Patients with type A HE often have no obvious anatomical abnormalities in their brains, but 38–50% of patients have brain edema, which may be a secondary change of the disease. Hypertrophy and hyperplasia of the original plasma astrocytes in gray matter and subcortical tissue can be found in patients with type C HE. Patients with longer course of the disease will exhibit brain atrophy (especially in patients with alcoholic cirrhosis) of different degrees, thinning of the cerebral cortex, loss of neurons and nerve fibers, and deep cortical sheet necrosis, even the cerebellum and the base may also be involved.

2.5.5 Clinical Epidemiology

The majority of patients with cirrhosis may have different degrees of HE at some stage in the course of the disease. The incidence of HE in patients with liver cirrhosis is at least 10–50% in mainland China while the incidence of Post-TIPS (transjugular intrahepatic portosystemic shunt) HE is 25–45%. If patients with chronic liver disease have HE, the outcome is poor; the one year survival rate is lower than 50% and the 3 year survival rate is less than 25% [248, 249].

The incidence of mild HE is 39.9% in mainland China in patients with liver cirrhosis, 24.8% in patients with Child-Pugh A, 39.4% in patients with Child-Pugh B, and 56.1% in patients with Child-Pugh C. The incidence of mild HE is not significantly associated with cirrhosis; however, with the increased degree of decompensated liver cirrhosis, the incidence of mild HE increase. Several studies have found that the incidence of depression and anxiety in patients also increased, With the increase of liver function damage, the incidence also increased, and the outcome is poor [250, 251].

2.5.6 Clinical Manifestation and Stages of HE

The clinical manifestations of HE vary, because of the difference in the nature of underlying disease, the degree of liver cell damage, the speed of injury and incentives. They are not specific to HE compared with other metabolic encephalopathies. Early pathological changes of HE are mild HE. The neuropsychological and intelligence tests detect mild form of HE, which exhibit no clear clinical symptoms and often develop symptomatic HE. The main clinical manifestations seen in acute liver failure induced by type A He are rapid-onset jaundice, bleeding, decrease in prothrombin, and eventually, change in mental status that can start as mild confusion but progress to coma and even death.

Type C HE is characterized by chronic recurrent episodes of changes in personality and behavior [252], stupor and coma, which is often accompanied by increased muscle tone, hyperreflexia, hepatic flap, ankle clonus or positive Babinski sign and nervous system abnormalities. Most patients in the early stages relapse, but then their symptoms become persistent. HE often has a variety of risk factors such as consuming a high-protein diet or discontinuing treatment of HE. Patients with type C HE not only have the clinical manifestations of encephalopathy, they also have chronic liver injury, cirrhosis and other clinical manifestations [226]. Observation of encephalopathy dynamic changes is beneficial for early diagnosis, treatment and analysis of treatment efficacy. HE can be graded and quantified according to the degree of disturbance of consciousness, nervous system performance and EEG changes. According to the 2009 edition of the “Consensus on the Diagnosis and Treatment of Hepatic Encephalopathy” in China, HE is divided into 0–4 periods, but each period can be overlapping or distinct but each period can be overlapping (Table 2.4). At present, scholars have stressed that the occurrence of HE is a continuous progression of the disease and should be viewed as a continuum of a wide

Table 2.4 Clinical staging of hepatic encephalopathy

Staging	The degree of cognitive dysfunction, personality and abnormal behavior	Signs of the nervous system	Changes in EEG
Grade 0 (MHE)	No change in behavior and personality, but minor abnormalities on psychological or intelligence tests.	No	Normal α rhythm
Grade 1	Patients with mild personality changes or behavioral disorders, such as euphoria or depressed state; urinary and fecal incontinence accurate response, but articulation is slow and unclear; impaired concentration or sleep cycle inversion.	asterixis	α and θ rhythms
Grade 2	Patients with sleep disorders and mental disorders, slow response, disorientation, decreased computing power and understanding, unclear speech, writing disorders, behavioral abnormalities, obvious sleep time inversion, even hallucinations, fear and manic. Involuntary movement or movement disorders.	Tendon hyperreflexia, increased muscle tone, positive sign of ankle clonus, Babinski and asterixis	Persistent θ, occasional δ
Grade 3	Patients with lethargy and insanity and can be awakened and answer questions, but often be unconscious or illusory.	Tendon hyperreflexia, increased muscle tone, positive sign of ankle clonus, and asterixis	Transient complex waves with spike and slow waves
Grade 4	Patients with loss of consciousness and cannot be awakened Shallow coma patients with pain response Deep coma	Shallow coma patients with positive sign of ankle clonus, tendon hyperreflexia and increased muscle tone deep coma patients with no response to stimulation	persistent δ, many complex wave with spike and slow waves

range of neuropsychiatric abnormalities, rather than isolated clinical stages. According to the traditional West Haven criteria diagnosing grade 1 HE is based on clinical signs and physician assessments, resulting in diagnostic criteria confusion [253]. To facilitate international communication and guide clinical practice, in 2011 the International Society of Hepatic Encephalopathy and Nitrogen Metabolism (ISHEN) proposed a new five-grade method for HE: HE (West Haven Level 0), covert HE (West Haven Level 1 HE), mild overt HE (West Haven Level 2 HE), severe overt HE (West Haven Level 3 HE) and coma overt HE (West Haven Level 4 HE) [228] (Fig. 2.3). Covert HE is diagnosed by a variety of neuropsychological and intelligence tests; the evaluation of overt HE widely uses the modified West Haven semi-quantitative grading table for the analysis of patients with neuropsychiatric state (Table 2.5), the Glasgow coma scale for the analysis of the degree of consciousness of patients, and the simple HE Severity Rating Scale for the disease

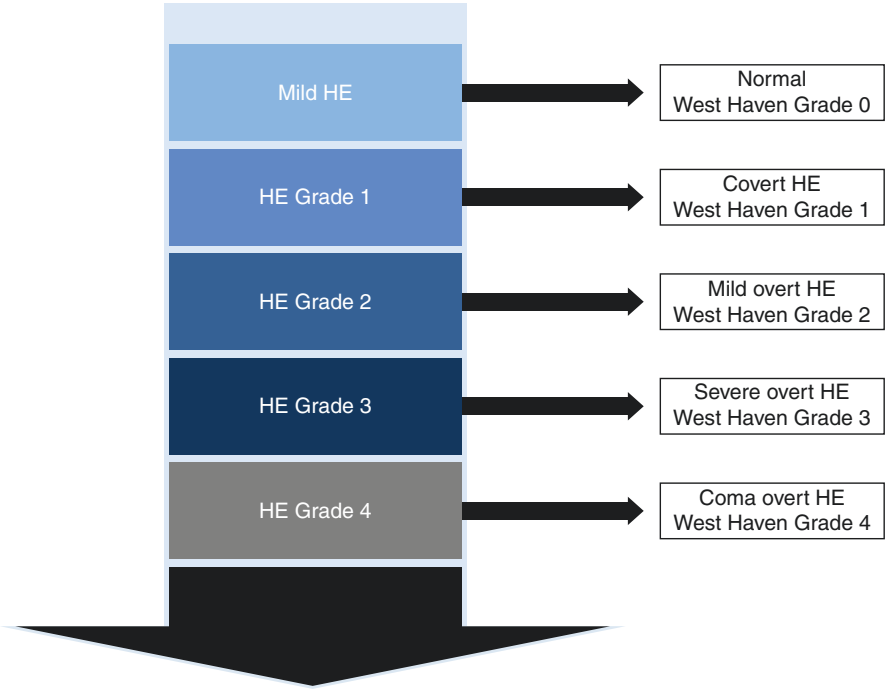

Fig. 2.3 HE Classification of Ammonia Metabolism Association and West Haven

severity in the patients. The HESA (hepatic encephalopathy scoring algorithm) scoring method introduced by Hassanein et al. may be more objective, accurate and operable in reflecting neuropsychiatric status [254].

2.5.7 Laboratory and Auxiliary Tests

In addition to abnormal liver function (such as increased bilirubin, enzyme bile separation, and decreased prothrombin activity) commonly used auxiliary examinations for HE diagnosis include: determination of ammonia, amino acid analysis of plasma and CSF, psychological intelligence test, neurophysiological test, electroencephalogram and neuroimaging.

2.5.7.1 Ammonia

The normal level of fasting venous ammonia is 6–35 μg/L (serum) or 47–65 μg/L (whole blood) and arterial ammonia concentration may be 0.5–2 times that of venous ammonia. Generally, the determination of arterial ammonia is common in clinical practice than intravenous determination; however, if venous blood has been transported on ice box and detected in a timely manner after proper collection, the

Table 2.5 West Haven Semi-quantitative classification

Grade	Symptom	Operation definition
0	No abnormality seen	
Minimal HE	Abnormal results on neuropsychological tests	Two or more tests PHES>2SD
	Normal examination	ICT > 5
	Abnormal results on psychometric tests	CF ≤ 39 Hz
1	Trivial lack of awareness	Respond 7 animal names within 120 s
	Euphoria or anxiety	Normally oriented in time and space
	Shortened attention span	
	Impaired performance of addition	
2	Lethargy or apathy	Disoriented for time (at least 3 of the following are wrong: day, week, month, season, or year)
	Minimal disorientation in time or place Subtle personality change Inappropriate behavior	
	Impaired performance of subtraction	Normally oriented in space
3	Somnolence to semi-stupor	Disoriented for space (at least 3 of
	Gross disorientation	the following wrongly reported: country, state/ region, city or place)
		Disoriented for time
		GCS 8–14
4	Coma (does not respond to verbal or noxious stimuli)	GCS < 8

PHES psychometric hepatic encephalopathy score, *ICT* inhibitory control test

result is expected to be as effective as arterial detection. Ammonia levels are increased in type B and C HE, but are normal in type A HE. Thus, HE cannot be ruled out based on having a normal ammonia level. The increased level of ammonia was reported to be associated with the degree of type A HE, but significant overlaps in different clinical stages of patients were also found [255, 256]. Therefore, ammonia detection is not routinely recommended in the diagnosis of HE. Notably, we need to rule out falsely elevated levels of ammonia caused by lab error, renal failure, complete parenteral nutrition, gastrointestinal bleeding, the use of steroid hormones and other extrahepatic factors.

2.5.7.2 Amino Acids in Plasma and Cerebrospinal Fluid

The Fischer ratio (BCAA/AAA) is used as a marker of HE, the plasma BCAA levels decrease while AAA levels increase; resulting BCAA/AAA: < 1 (normal >3). It was reported that the concentration of glutamate in CSF in HE patients is increased compared to healthy controls. The concentrations of phenylalanine and tyramine in CSF were also significantly increased, and the level of phenylalanine was closely related to the degree of HE [257]. Recently, it was reported that H–nuclear magnetic resonance spectroscopy could select biomarkers for these diseases, such as in patients with HE [258], but this is not commonly used clinically because the

specialized equipment required for the analysis is not available in the general laboratory.

2.5.7.3 Neuropsychological and Intelligence Tests

The characteristic manifestations of cognitive dysfunction in patients with covert HE are lack of attention, working memory problems, and deficits in executive function. Therefore, various intelligence tests are used to assess the subtle changes in a patient's cognitive or precise movement, which is important for the diagnosis of covert HE, but not for overt HE. Of the various intelligence tests, psychometric HE score (PHES) and repeatable battery for assessment of neuropsychological status (RBANS) are recommended by the WCOG. The PHES includes a number connection test A (NCT-A), number connection test B (NCT-B), digital symbol test (DST), trajectory rendering test (line tracing test, LTT) and serial dotting test. The best known and validated Portal Systemic Hepatic Encephalopathy Score (PHES) tests are relatively simple, easy and cheap for the diagnosis of minimal HE; however, it was found that the result of this time consuming method was influenced by the patient's age, gender and education level [259].

At present, computer-aided psychological tests such as information and communication technology (ICT), cognitive drug research test (CDR), and critical flicker fusion test (CFF) are not influenced by the factors mentioned above and easily operated, which can be used as an alternative choice for pen and paper tests. ICT with sensitivity 87% and specificity 77% was one of the most commonly used tests to diagnose minimal HE. CFF was originally used to detect the critical flicker frequency of alert patients, reflecting brain conduction dysfunction. Based on a Spanish study of 217 cases, including patients with cirrhosis and healthy controls, CFF was a sensitive method to diagnose covert HE with sensitive, simple and reliable advantages [260, 261]. Because the diagnosis of minimal HE has just started, the related diagnostic value still needs to be further evaluated.

2.5.7.4 Neurophysiological Tests

Electroencephalography (EEG)

An abnormal EEG is often observed before biochemical abnormalities or mental abnormalities [262]. The main abnormalities by EEG are slowed rhythm, sporadic or universal θ wave (4–7 times/s) and the occasional α wave (1–3 times/s). With the deepening of consciousness, symmetrical δ-wave and three-phase waves appear on both sides simultaneously. This change usually occurs on both sides of the forehead and the top, gradually moving backwards. Although these EEG changes are not specific to HE and can appear in uremic encephalopathy and other metabolic encephalopathy, the severity of changes have a good correlation with clinical stages of HE. Computer analysis of EEG frequency distribution, such as artificial neural network-expert system (ANESS) and short epoch dominant activity cluster analysis (SEDACA), is more objective and valuable in diagnosing minimal HE than conventional EEG [263].

Evoked Potential Tests

There are many kinds of evoked potential tests, including visual evoked potential (VEP), brainstem auditory evoked potential (BAEP), somatosensory evoked potential (SSEP) and endogenous event evoked potential (event-related potentials, ERPs) P300, of which the P300 is the most sensitive test for the diagnosis of HE. Compared with intelligence tests, neurophysiologic tests, independent of age and education background, are more objective. However, they are only used in clinical studies and are limited by equipment, and professional operation.

2.5.7.5 Imaging Tests

Based on cerebral CT and MRI, brain edema can be found in patients with type A HE while brain atrophy in the frontal cortex, and the T1-weighted signal enhancement in the globus pallidus can also be found, which may be associated with manganese deposition. Detected by H-magnetic resonance spectroscopy (H-MRS), the metabolic changes of HE patients in the brain include increased levels of glutamate and glutamine, and decreased levels of inositol, taurine and choline [263]. Using fluid attenuation inversion recovery (FLAIR) and diffusion weighted imaging (DWI) techniques, diffuse T2-weighted signal enhancement is found in the hemisphere white matter and corticospinal tracts, which may be associated with cerebral ischemia. However, the sensitivity and specificity of the above-mentioned imaging abnormalities remain unknown, and the correlation with HE staging is not clear. Therefore, the main significance of cranial nerve imaging is to exclude cerebrovascular accident, intracranial tumors and other diseases, rather than diagnose HE.

2.5.8 Diagnosis and Differential Diagnosis

There is no gold standard diagnostic criteria of HE, and diagnosis is mainly based on the exclusion of other diseases. But one should consider the following five factors [232]:

1. Hepatic and Nonhepatic Diseases that May Lead to HE

Several forms of hepatic diseases may lead to different kinds of HE. Type A HE is caused by acute hepatic failure, but without chronic hepatic disease. Type B is caused by PSS, but without any history of hepatic diseases. Type C is caused by serious hepatic diseases and/or widespread PSS, such as cirrhosis, liver cancer, post-TIPS and so on.

2. Physical Signs and Psychiatric Symptoms

Psychiatric symptoms can be found such as change of mood and personality, Dementia, behavior disorder and disorientation. Drowsiness alternating with excitability, hypermyotonia, asterixis, ankle clonus, insanity and coma are physical signs could be present in progressed patients.

3. Additional Symptoms and Signs

Some patients may lack related physical signs and psychiatric symptoms, but have deficits in ability of learning, understanding, concentration, and quick verbal response.

4. Presence of Risk Factors for HE (Type B and C)

Upper gastrointestinal hemorrhage, ascites tapping, excessive diuresis, high protein diet, medicine (such as sedatives) and infection could lead to HE. Previous HE symptoms could be helpful for the diagnosis. Type A usually does not have any risk factors.

5. Exclusion of Other Diseases

Metabolic encephalopathy includes ketoacidosis, hypoglycemia, uremia, pulmonary encephalopathy, serious electrolyte disturbances and toxic encephalopathy. Nervous system diseases include intracranial hemorrhage, infection or tumors, mental diseases and excessive use of sedatives [264]. But one should also watch out for the coexistence of HE in these situations.

An overt HE should be considered if (1), (3), (4), and (5) coexist; and covert HE is based on (2), (3), (4), and (5) [228]. Then, based on the degree of neuropsychiatric symptoms, determine the stage of HE, or for HE classification refer to the West Have semi-quantitative classification table or ISHEN scores. The flow chart of diagnosis is shown in Fig. 2.4.

2.5.9 Treatment of HE

HE is a complex metabolic disorder caused by many factors and comprehensive measures should be taken to cure it from different aspects. According to the clinical type, inducements and the severity of the disease, different plans of treatment should be designed for HE. At present, the treatment of overt HE generally includes the following aspects: (1) supportive treatment; (2) identification of possible concurrent encephalopathy and removal of other precipitants; (3) cause of treatment; and (4) empirical treatment (Fig. 2.5).

2.5.9.1 Supportive Treatment

Nutritional Therapy

The point of nutritional therapy is to promote anabolism, inhibit catabolism, and maintain a positive nitrogen balance, rather than simply limiting protein intake. To reduce the source of ammonia, it has been suggested that patients with HE should limit protein intake. In critically ill patients, it has been suggested that they should stop all protein intake and, after the disease improves, gradually increase

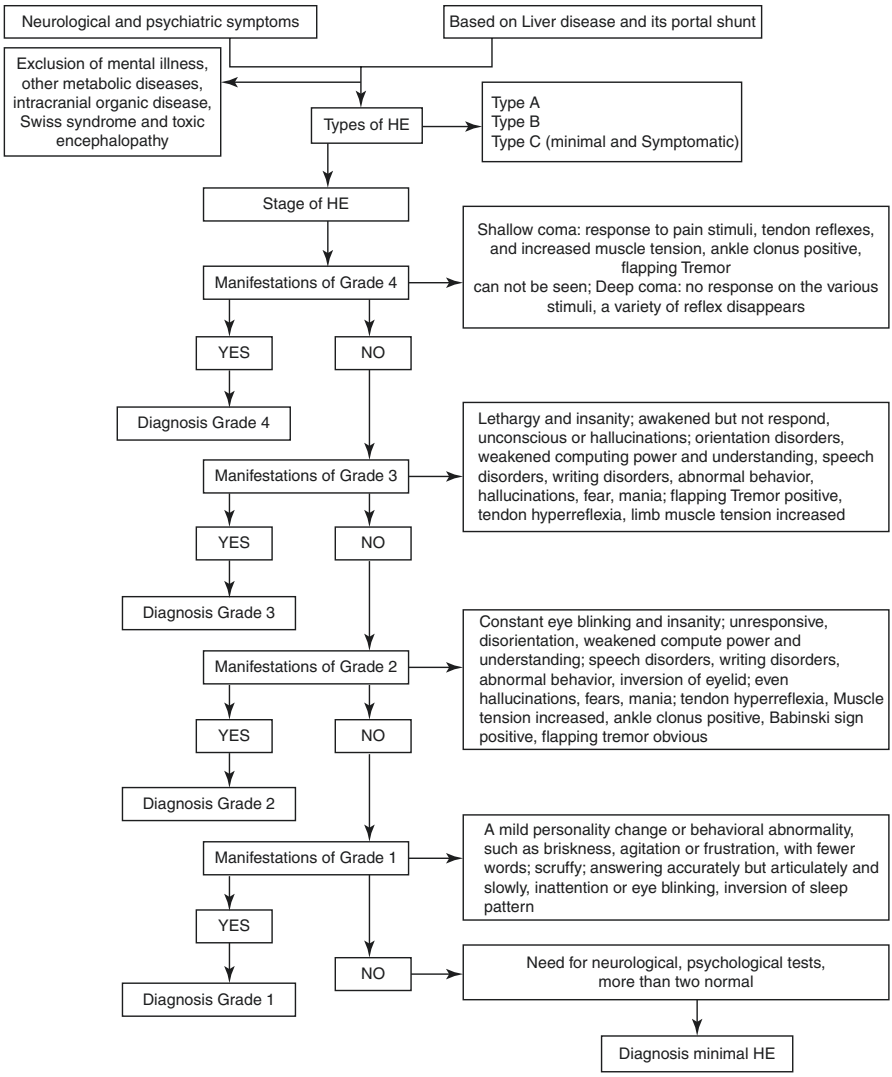

Fig. 2.4 The flow chart of diagnosis of HE

protein intake to the maximum clinical tolerance. These recommendations are now being questioned because most cirrhotic patients are malnourished and all long-term protein-restricted diets increase the severity of malnutrition. In addition, a negative nitrogen balance increases mobilization of skeletal muscle, resulting in a reduction in ammonia metabolism that may increase blood ammonia levels. Recent studies have shown that normal ingestion of protein 1.2 g/(kg • d) can also improve the health-related quality of life (HRQOL), especially in MHE [265]; and have no adverse effects on the recovery of blood ammonia and HE

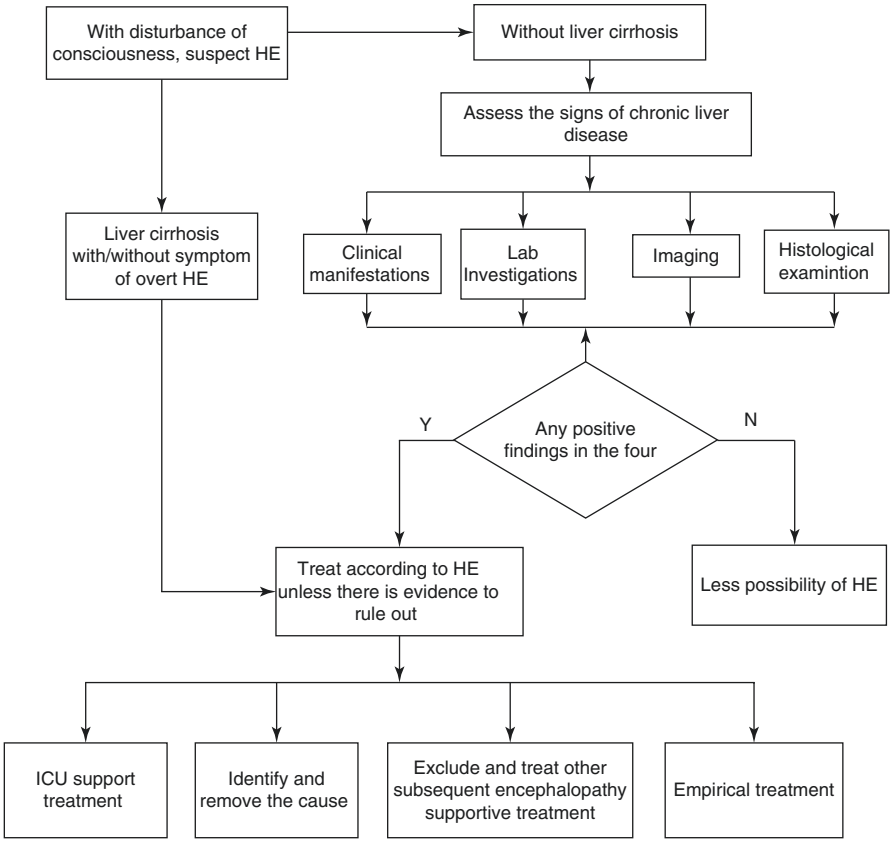

Fig. 2.5 Treatment schemes for overt hepatic encephalopathy

compared with the restricted protein intake. According to the guidelines of the European Society of Enteral Nutrition in 2009, the intake of protein should be guided by the following principles: patients with acute phase HE on the first day should be put on a prohibited protein diet and given glucose to ensure energy supply and those who cannot eat food may be fed through a nasogastric tube without short-term fasting; patients with chronic HE do not need to fast and their intake of protein should be 1–1.5 g/(kg • d); oral or intravenous use of BCAA and essential amino acid preparations can be administered to adjust the balance of AAA/BCAA, promote the balance of nitrogen, and also reduce the risk of HE recurrence [266]; probiotics and prebiotics can enhance the body's tolerance to protein; plant protein is superior to animal protein because it contains methionine, has less AAA, and more BCAA, but it also contains cellulose, which is conducive to maintain the normal flora in the colon and acidize the intestinal tract, shortening the transit time of the colon and reducing absorption of ammonia. The above points need further verification.

Other Supportive Treatments

Additional supportive treatments include: maintaining adequate hydration, electrolytes and acid-base balance; ensuring an energy supply of 30–35 kal/(kg • d), which should be composed of 50–60% sugar, 20–30% protein, and 10–20% fat; administering appropriate vitamins and trace elements; treating for hypokalemia, hyperkalemia, hyponatremia, hypocalcemia, hypomagnesemia and metabolic alkalosis as needed; strengthening the basis of treatment with the appropriate infusion of fresh plasma or albumin, increased plasma colloid osmotic pressure; treating for hypoxemia and cerebral edema; and preventing and treating any bleeding and bacterial infection.

2.5.9.2 Removal of Precipitents

Type C HE has a variety of precipitents. Actively finding and eliminating triggers can effectively prevent the development of HE, such as esophageal variceal bleeding that can develop into HE. Active hemostasis, anemia correction, and removal of intestinal blood are also conducive to controlling HE. In addition, active control of infection, correction of water and electrolyte imbalance, elimination of constipation and improving renal function are essential to control HE. Anesthetics, painkillers, sedatives, sleeping pills, and other drugs should be strictly controlled. Patients with mania or convulsions can reduce the use of diazepam and scopolamine, and the frequency of administration can also be reduced for promethazine, chlorpheniramine, and other antihistamines.

2.5.9.3 The Empirical Measures Targeting the Pathogenesis of HE

Reducing the Intestinal Ammonia and Generation and Absorption of Other Harmful Substances

(a) Enema or promoting fecal elimination

Toxic substances causing HE mainly come from the intestine. Thus, in order to prevent and control HE, it is very important to clean the intestinal tract to reduce the generation and absorption of ammonia and other toxic substances. Saline or weak acidic solution enemas (such as a dilute acetic acid solution), or oral or nasal feeding of 25% magnesium sulfate (30–60 ml) can be used to clear intestinal hemorrhage, intestinal impaction, and other toxic substances. An enema composed of non-absorbable lactulose (300–500 ml) plus water (500 ml) is also useful, especially when applied for type B HE. A recent clinical trial suggested that polyethylene glycol was more effective than the current standard first-line therapy in treating these patients [267]. Another two studies showed that polyethylene glycol was more effective than the standard lactulose therapy in treating patients with acute HE by cirrhosis [268, 269]. Other available drugs include pear liquors, mannitol, rhubarb, and so on, but excessive use of these substances may lead to dehydration and aggravate HE.

(b) Non-absorbable disaccharides

Non-absorbable disaccharides include lactulose and lactitol. Lactulose, a kind of synthetic ketone disaccharide, cannot be broken down in the stomach and small intestine due to a lack of enzymes that can break down galactose in the digestive tract. After entering the colon, lactulose can be broken down into acetic acid and lactic acid with the help of gut bacteria, leading to a reduction in the colonic pH and inhibition of the absorption of ammonia in the intestine. These non-absorbent disaccharides are decomposed into organic particles in the intestinal tract, which can increase the osmotic pressure of the intestine, and their acidic products stimulate the intestinal wall and can slightly promote intestinal excretion. These non-absorbent disaccharides, acting as prebiotics in the intestine, can inhibit the growth of bacteria, which can produce ammonia and urea, finally reducing the production of ammonia and reversing low-grade cerebral edema when combined with rifaximin [270]. However, probiotics can benefit patients in the long-term [271]. Oral or nasal feeding (15–30 mL, 2 or 3 times daily) was recommended to adjust the daily defecation appropriately, about 2–3 times daily. Main adverse reactions include abdominal discomfort, abdominal distension, abdominal pain, loss of appetite, nausea, vomiting, and diarrhea. Lactulose can even be used in patients with diabetes or lactose intolerance when the purity of non-absorbable disaccharide was high ($\geq$ 98%), but is not used in patients with intestinal obstruction.

Numerous randomized controlled studies showed that lactulose or lactitol can significantly alleviate overt HE and improve the patient's cognitive function and quality of life [272, 273]. Lactulose is still the first-line therapy of anti-HE, although its effect on improving the survival rate of patients is uncertain.

(c) Antibiotics

Antimicrobial agents can be used as a substitute for non-absorbable disaccharides in treating acute and chronic HE. In the past, oral aminoglycoside antibiotics, such as neomycin, which are rarely orally ingested, were used to inhibit the overgrowth of bacteria in the intestine. However, recent randomized placebo-controlled studies have shown that neomycin may not benefit patients with HE compared with placebo-treated patients and that long-term use of neomycin may lead to increased ear and renal toxicity risk and impair the function of small intestinal mucosa [274]. Metronidazole can inhibit anaerobic bacteria in the intestine and alleviate HE, but long-term use may lead to disruption in the intestinal flora, gastrointestinal discomfort, or neurotoxicity. Rifaximin, a derivative of rifamycin with a broad-spectrum, has a potent inhibitory effect on intestinal bacterial growth, is a minimally-absorbed oral antibiotic and only plays a role in the gastrointestinal part. Administration of rifaximin (550 mg, twice a day) can significantly prevent the occurrence of HE compared with placebo-treated patients [275–277]; rifaximin was equivalent to or better than lactulose and neomycin in treating patients with chronic HE [278]. A study indicated that rifaximin-α in combination with lactulose was a cost-effective therapy for patients who had experienced at least two prior overt HE episodes [270],

and this therapy could also improve the driving ability of patients with covert HE without toxicity to the auditory nerve and renal function [274, 277]. Thus, rifaximin has been recommended by the US Food and Drug Administration (FDA) for the prevention of recurrent HE. The efficacy of rifaximin and relation between long-term use of rifaximin and intestinal flora in the treatment of HE needs to be further investigated. However, a recent study in mice showed that rifaximin beneficially alters intestinal ammonia generation by regulating intestinal glutaminase expression in MHE [277]. The study may provide a new opportunity to study intestinal flora in the treatment of HE.

(d) Probiotics

Microecologics with *Bifidobacterium* and *Lactobacillus* can regulate intestinal flora structure to inhibit the growth of bacteria that produce ammonia and urease. In combination with prebiotics, microecologics can reduce the production and absorption of intestinal ammonia and other toxic substances [279, 280]. In a recent open-label study, 190 patients with cirrhosis were randomized to three groups and treated with lactulose (30–60 mL daily), probiotic capsules, or with both drugs. After a month of treatment, patients with HE showed better results in the neuropsychological test, P300 auditory evoked potentials, and blood ammonia. However, there was no difference in the therapeutic effect among the three groups [281, 282].

Promoting Transformation and Metabolism of Ammonia

Clinicians commonly use sodium glutamate, potassium glutamate, arginine hydrochloride and potassium magnesium aspartate, but the exact efficacy is highly controversial at present and effective drug reduced ammonia is described below.

(a) L-Ornithine-L-Aspartate

LOLA, a dipeptide, can lower blood ammonia by promoting ammonia consumption and the synthesis of urea, glutamic acid and glutamine in brain, liver and kidney [283, 284]. Ornithine, a substrate of the ornithine urea cycle, can increase activity of carbamyl phosphate synthetase and ornithine carbamyl transferase, and promote urea synthesis. N-methyl-D-aspartate (NMDA) is a substrate of glutamine synthesis, and the conversion of glutamic acid to glutamine in the body can remove blood ammonia [285]. NMDA is also involved in nucleic acid synthesis in liver cells and indirectly improves the metabolism of the Krebs cycle process in liver cells to facilitate the repair of liver cells. Clinical studies show that, compared with a placebo group, 20 g/days LOLA intravenously could noticeably reduce fasting blood ammonia (FNH3), postprandial blood ammonia, and improve the mental status of patients with HE [286]. Patients with oral LOLA also had improved HE examination results for the digital connection test, the flapping tremor, and EEG results [287]. In addition, glycerol phenylbutyrate (GPB) can safely reduce the incidence of HE as well as ammonia in patients with cirrhosis and HE. The results showed that GPB had therapeutic potential in this population [288].

(b) Zinc

Zinc is an important cofactor in urea cycle enzyme catalysis. A study in HE patients showed that serum zinc concentration is reduced, and showed a negative correlation with the blood ammonia concentration; the serum ammonia level is much lower after zinc supplementation in patients, and HE can be improved in some patients. A new study suggests that antioxidant and zinc supplementation can improve MHE in patients with liver cirrhosis [289]. Oral zinc preparation can also reduce absorption of divalent cations such as manganese in the intestine; however, it has not been determined if zinc has a positive therapeutic effect on HE.

(c) Sodium benzoate

Sodium benzoate can lower the blood ammonia concentration by activating the urea cycle for ammonia detoxification and promoting urinary ammonia. Randomized controlled studies showed that sodium benzoate had the same efficacy as lactulose in treating patients with HE. The recommended sodium benzoate dose is 5 g twice a day; nevertheless, few patients can tolerate this dose because of its high gastrointestinal side effects [232]. A recent study showed that Tranilast could protect patients from thioacetamide-induced acute liver injury and alleviate HE [290].

Pseudo Neurotransmitter Antagonism

Endogenous Bz analogues combine with the inhibitory neurotransmitter GABA receptor to depress the action on the CNS, and is one of the occurring hallmarks of HE pathogenesis. A large-scale clinical study on 560 HE cases showed that the improvement rate in brain function in treatment and control groups were 15% and 3%, respectively [291]. The study showed that treatment of HE with receptor antagonists such as fluorine marcie is feasible. A meta-analysis which included 13 case-control studies of 805 patients show that fluorine marcie can noticeably improve HE, but didn't show any long-term benefits or improve patient survival rate. So fluorine marcie should only be considered for HE patients who had used Bz. Although the reduction of dopamine neurotransmitter activity is also one of the pathogenesis, the application of bromocriptine, levodopa, has been unable to bring more benefits besides partly improving symptoms of patients.

Improved Amino Acid Balance

Oral or intravenous infusion with a BCAA-based amino acid mixture can theoretically correct an imbalance in amino acid metabolism and control false neurotransmitter formation in the brain [292]. A meta-analysis which included five studies showed that intravenous BCAA did not reduce the mortality rate of HE. Three studies with BCAA did not reduce the mortality rate of HE; however, two larger studies (randomized controlled study about patients with liver cirrhosis in 174 cases and 622 cases, respectively) show that the application of BCAA not only reduced the

occurrence of HE and liver failure, but also improved the nutritional status, liver function and survival rate in patients. Another study showed that BCAA could stimulate liver cell regeneration thus reducing the occurrence of liver failure. Supplementation with a BCAA-rich amino acid mixture showed improved restoration of the patients' positive nitrogen balance, and increased the patient's susceptibility to protein food, improving cerebral perfusion [293].

Other Therapies

Considerable progress has been made to understand treatment of HE, studies of basic and clinical research are underway using newly discovered treatment strategies, such as Toll-like receptor 4 antagonists (with the ability to reduce systemic inflammation and oxidative stress) as well as Non-steroidal anti-inflammatory drugs (Ibuprofen) [286, 294], NMDA antagonists, anticholinesterase.

However, research using gene therapy should not be ignored [295]. After TIPS, LOLA can significantly reduce the increase of venous ammonia concentration in patients with HE [296]. And the positive dietary intervention can significantly reduce the incidence of HE [297]. For patients with refractory HE, embolization of PSS is a safe and effective treatment strategy [298].

2.5.9.4 Treatment of Underlying Disease

Improving Liver Function

Antiviral treatment with nucleos(t)ide analogues can reduce or eliminate liver inflammation and necrosis, promote the regeneration of liver cells, and help restore the functions of hepatic metabolism and detoxification in chronic liver failure caused by hepatitis B virus.

Artificial Liver Support System

Artificial liver support systems can be divided into three types including non-biological type, biological type and mixed type. The non-biological liver support system is the most widely used type, and consists of hemodialysis, hemofiltration, plasma exchange, blood perfusion, plasma adsorption, and the molecular adsorption recirculation system (MARS) [299]. The artificial liver support system can replace the partial function of the liver, remove the poison accumulated in the body, create conditions that allow for the regeneration of liver cells and provide enough time to wait for liver transplantation for patients with HE. An artificial liver support system can be used to treat acute and chronic HE, but patients with overt stage 2 HE should be especially careful with plasma exchange. Liver transplantation remains the only promising therapy for patients with an acute liver failure or end-stage liver disease.

Liver Transplantation

Liver transplantation is an effective means for all kinds of persistent and severe HE; however, in patients with HE there is a significant increase in mortality among

patients awaiting liver transplantation [300]. Recently, it has been reported that cognitive function was not fully recovered after liver transplantation in some patients with severe HE [301]. The key point in improving the prognosis of HE is early recognition and timely treatment. Active treatment should be given when diagnosing covert HE.

Blocking Portosystemic Shunting

Theoretically, for patients with serious PSS, interventional therapy, surgery or permanently/temporarily and partially/totally blocking the PSS can improve the patient's symptoms. The use of this therapy should be carefully weighed because it can increase the risk of gastrointestinal bleeding in case of portal hypertension.

The Treatment of Covert HE

Covert HE has gained an increasing amount of attention in recent years. Patients with covert HE do not exhibit obvious signs and symptoms; however, their quality of life is reduced because of reduced operational ability or sleep disorders. Without treatment, covert HE will progress to overt HE over time. The population with high risk should be examined and treated early, especially those engaged in potentially dangerous occupations. The following solutions can be referred to: (a) adjusting dietary structure (vegetable protein is the main intake); (b) oral administration of lactulose (15-30 ml, 2–3 times daily); (c) oral administration of rifaximin (550 mg, twice a day); (d) oral administration of LOLA (6 g, 3 times a day); (e) oral administration of BAAA; and (f) oral administration of probiotic preparations [271, 275, 289].

2.5.10 Prospect

Although medical technology has made great progress and the research into HE is also increasing in recent years, the pathogenesis of HE is still unclear. Due to a lack of specific methods, combination treatment is still the main therapy for HE. It is generally believed that the onset of HE may be a result of the synergistic effects of many factors. Therefore, it is difficult to implement and draw convincing conclusions from randomized controlled trials with a single intervention targeting a specific pathogenesis and risk factor. Ongoing issues remain, such as standardizing the research design of HE treatment and evaluating the efficacy of HE treatment more scientifically and objectively. Some clinical studies may bring new hope for HE treatment by new ongoing strategies of targeted systemic inflammation, oxidative stress, and neurosteroids. In addition, the key point in improving the prognosis of HE is early recognition and timely treatment. Active treatment should be given when diagnosing covert HE. It is difficult to popularize the cognitive dysfunction detection methods of latent HE. So the key and difficult point is to develop new method of assessments for clinicians in the future.

2.6 Hepatopulmonary Syndrome

Jia Shang

2.6.1 Definition

Hepatopulmonary Syndrome (HPS) is a syndrome of shortness of breath and hypoxemia induced by vasodilation in the lungs of patients with a variety of acute and chronic liver disease. Essentially primary liver disease, pulmonary vasodilation and arterial oxygen lack of co-triad constituted. Due to abnormal increase of vasodilators、ventilation/blood flow disproportion and pulmonary hypertension caused by liver disease, the hypoxemia ($PaO_2 < 9.33$ KPa) (70 mmHg) is included in HPS.

2.6.2 Introduction

When Fluckiger reported a 37-year-old syphilis female patient as early as in 1884, he described cirrhosis, cyanosis and clubbing at the same time, while he was not aware of the intrinsic relationship between these clinical manifestations [302]. In 1935, Snell reported decreased arterial oxygen saturation (SaO_2, less than 94%) with abnormal hemoglobin in 38 patients with liver parenchymal lesions and biliary obstruction, and 3 years later, he proposed that such a phenomenon was associated with decreased affinity of O_2 with hemoglobin. In 1956, Rydell and Hoffbauer reported the detailed clinical diagnostic and treatment process of a 17-year-old male with "juvenile cirrhosis", and found multiple arterial-venous anastomoses in the lungs during autopsy, which he thought contributed to clinical cyanosis mainly. This provided a histological basis for the patient, and people conducted a large amount of studies thereafter.

In 1966, Berthelot et al. injected opaque glue into the pulmonary vascular beds at the time of biopsy after the patient's death for the first time, and he found abnormal small arterial dilation in the lungs of the patient with cirrhosis, which he termed lung spider nevus. The term hepatopulmonary syndrome (HPS) was first proposed by Kennedy and Knudson in 1977 [303]. After nearly 20 years of studies in a large number, people gradually developed a clear understanding of the mechanisms underlying its pathogenesis. In 1988, Eriksson used the term *functional hepatopulmonary syndrome* for the first time. In 1989, the famous liver disease expert Sherlock formally used this diagnostic term in his monograph *Hepatobiliary Diseases*, which has been recognized by many scholars [304].

2.6.3 Epidemiology

HPS can occur in patients of any age groups, and various literature reports show conflicting incidences of HPS in patients with cirrhotic portal hypertension, with the

average incidence of various chronic liver diseases being about 5–29%. The incidence of cirrhosis in patients is high, and 30–70% of patients can additionally develop mild arterial hypoxia and 12–28% develop arterial hypoxia. In the study by Binay on Indian cirrhotic populations arising from hepatitis B mainly, the incidence of this disease is relatively low (6.7%) [305]. The differences in incidence were mainly attributable to the different diagnostic criteria adopted.

Schenk et al. studied the incidence of HPS by performing transthoracic contrast echocardiography (TTCE), pulmonary function tests and blood gas analysis on 98 patients with cirrhosis patients. The results showed that the incidence of HPS patients in whom alveolar-arterial partial pressure of oxygen ($AaPO_2$) was used as an indicator of hypoxemia was significantly higher than those in whom arterial partial pressure of oxygen (PaO_2) was used [306]. When arterial partial pressure of oxygen (PaO_2) was reduced to reflect hypoxemia, HPS incidence was 19% when <80 mmHg and 15% when <70 mmHg, respectively. While when increase in alveolar-arterial partial pressure of oxygen ($AaPO_2$) was used to reflect hypoxemia, the incidence of HPS was high, with 32% when >15 mmHg and 31% when >20 mmHg, respectively.

2.6.4 Etiology

HPS is most common in cirrhosis due to various causes. Pulmonary vascular abnormalities and arterial hypoxemia can occur in a variety of acute and chronic liver diseases, and this is true mainly when it comes to cirrhotic patients due to chronic liver diseases, especially cryptogenic liver cirrhosis, alcoholic cirrhosis, hepatitis-induced cirrhosis and primary biliary cirrhosis. Besides, HPS can also occur in chronic hepatitis, acute severe hepatitis, cholestasis, α-anti-trypsin deficiency [307], tyrosinemia, Wilson disease, and non-cirrhotic portal hypertension (such as idiopathic portal hypertension and schistosomiasis cirrhosis, etc.).

Arterial hypoxemia can also occur in extrahepatic portal vein occlusion. The observation of these patients suggests that portal hypertension may be the main factor for the pathogenesis of HPS. HPS can also occur in non-cirrhotic portal hypertension, and even cirrhosis- and portal hypertension-free chronic viral hepatitis. In 2000, Binay et al. found that patients with progressive liver failure with hyperdynamic circulation are most likely to suffer from HPS, while they did not find the correlation with the severity of liver cirrhosis.

2.6.5 Pathology and Physiology

HPS is, in essence, hypoxemia due to anomaly in pulmonary vascular dilatation and arterial oxygenation when liver disease occurs. Arterial hypoxemia occurs as the result of insufficient oxygenation by blood cells in the blood when blood flows through the lungs, or a proportion of blood fail to flow through the alveoli [308]. Since primary heart and lung diseases have been excluded when HPS occurs, the

abnormal pathways that red cells may pass through include: (1) passing through the pleural and hilar bronchial vessels while not reaching the alveoli; (2) blood flows directly into the pulmonary veins due to the high pressure portal system in the mediastinum, thereby bypassing the pulmonary circulation; (3) flowing directly into the pulmonary veins through the expanded alveolar capillaries or the pulmonary-venous fistula. Alveolar telangiectasia may be more important to the formation of hypoxemia, and existing study data show that the development of HPS is at least associated with the systemic hyperdynamic state, portal hypertension, hepatic encephalopathy, hepatorenal syndrome and pulmonary hypertension [308]. Therefore, it is believed that the main causes of HPS are systemic metabolism and hemodynamic disorders, and that it is involved in the formation of systemic metabolism and hemodynamic disorders, which is of important pathophysiological significance.

1. The basic pathological change of HPS is pulmonary vascular dilatation, which is manifested as:
   (a) dilation of anterior capillaries in a large number.
   (b) formation and opening of the pulmonary basilar arterial - venous communicating branches.
   (c) formation of pleural "spider mole", which is mainly manifested as dilation of anterior capillaries.

In autopsies, it was found that the basic pathological changes in patients with liver cirrhosis and other chronic liver diseases were extensive pulmonary vascular dilatation and arteriovenous communicating branches. Some people found the pathological changes through vascular shaping, with pleural vasodilation at the basal aspect of the lungs or the formation of subpleural spider nevus. Domestic professor Gu Changhai summarized these pathologic changes in 1997 as arterial dilation within the pulmonary acinus in a pattern of inhomogeneous distribution, thin-walled blood vessels, 60–80 μm in diameter, in the lower lobes of the whole lungs, extensive dilation of pulmonary vascular beds adjacent to the alveolar gas at the anterior capillary level, and significantly expanded pulmonary artery branches and pulmonary capillaries up to 160 μm in diameter. Electron microscopy showed thickened pulmonary capillaries, pulmonary arterial walls and the basal layers of small veins.

2. Factors that affect the dilation of blood vessels: the mechanisms underlying pulmonary vascular dilatation have not yet fully elucidated, and the possible influencing factors include:
   (a) Increased **activity of vascular dilators** Various acute and chronic liver diseases, liver cell failure and metabolic disorders, particularly reduced inactivation of vasoactive substances which can enter directly into the systemic circulation through abnormal anastomotic collateral vessels, result in disorder of the systemic hemodynamics and increased contents of vasodilators in the blood circulation. Just as visceral congestion in patients with portal

hypertension, they can act on the intrapulmonary vessels, causing pulmonary vascular dilatation and pulmonary congestion. Substances that cause vasodilation include glucagon, prostaglandin, vasoactive intestinal peptide, nitric oxide, angiotensin, bradykinin and endotoxin, etc.

(b) Reduced **vasoconstrictors or decreased sensitivity of intrapulmonary vascular beds to the endogenous vasoconstrictors**, such as norepinephrine, endothelin, atrial natriuretic peptide, vasopressin, serotonin and tyrosine, etc. The contents of the substances are not absolutely reduced because maybe their sensitivity is reduced. When chronic liver disease occurs, the anterior communicating branches of the originally closed non-functional capillaries may be opened, and a disorder occurs in the hypoxic pulmonary vascular systolic dysfunction which should have been normal, and it is only 75% of the normal state [309].

(c) Neurological **factors** Cirrhotic patients show sympathetic nerve hyperactivity, but after the formation of portal hypertension, their sympathetic nerve function may be damaged, which play an important role. Animals with portal hypertension often show abnormal pressure responses and reduced sensitivity of blood vessels to norepinephrine, resulting in increased cardiac output, and dilated pulmonary vascular volumes. Besides, hemodynamics within the lungs is also a manifestation of the body's hyperdynamics.

(d) Decreased **reactivity of intrapulmonary blood vessels to hypoxia** Recent inert gas dispersion tests show that cirrhotic patients with over two spider nevus are manifested as not only liver damage, but also decreased systemic intrapulmonary vascular resistance, decreased reactivity of blood vessels to hypoxia and dilated pulmonary vessels. However, it was also found using pulmonary angiography that in spite of the dilated vessels at the ending of arteries, the responses of vessels to oxygen were almost normal, which did not support this view.

(e) Intrahepatic angiogenesis or dysplasia may also be one of the factors for the formation of HPS.

To date, the mechanisms underlying pulmonary vascular dilatation caused by HPS is still unclear. However, long-term administration of intrapulmonary vasoactive substances can cause significantly increased intracellular cyclic adenosine monophosphate (cAMP) and/or cyclic guanosine monophosphate (cGMP), resulting in hypoxic pulmonary vasomotor dysfunction and pulmonary artery dilatation, which may be an important cause of this disease and also pulmonary manifestations of systemic hyperdynamic circulation. Due to the significant dilation of the pulmonary capillaries and the anterior capillaries, some of the blood around the capillaries in contact with the alveoli can still undergo exchanges with gases, while the central blood, due to the increased diffusion distance from the alveoli, leads to insufficient gas exchange, resulting in insufficient arterial oxygenation and thus a series of hypoxemic manifestations.

2.6.6 Mechanisms Underlying Its Pathogenesis

To date, the pathogenesis underlying the pathogenesis of HPS has not yet been elucidated. In view of the above pathophysiological changes and current studies, it is believed that the disease may be caused by insufficient ventilation, diffusion disorder, ventilation/blood flow imbalance and decreased oxygenated hemoglobin affinity, or the above factors in combination.

2.6.6.1 Insufficient Ventilation

Under normal circumstances, insufficient ventilation due to a variety of reasons causes insufficient oxygen inhaled into the alveoli and reduced blood oxygen exchanges, which can result in hypoxemia [310], such as chronic bronchitis, foreign bodies in trachea, atelectasis and respiratory muscular paralysis, etc. And the presence of insufficient ventilation in patients with chronic liver disease and cirrhosis or not is still controversial.

In 1982, Fujiwara studied the lung function in 22 patients with decompensated liver cirrhosis and reported that vital capacity (VC), functional residual capacity (FRC) and respiratory reserve volume (EVR) in the patients were significantly reduced, that R/T was mildly increased, and that there was no changes in 1 s forced expiratory volume (FEV1). Therefore, it was believed that mechanical compression and insufficient ventilation due to pulmonary interstitial edema in patients with liver cirrhosis was the main reason for impaired lung function. Subsequently, Edison et al. studied the pulmonary function of 21 patients with decompensated liver cirrhosis, and found that their VC, maximum ventilation volume (MVV), FRC, total lung volume, and R/T were significantly reduced, and they believed that patients with cirrhosis had obvious obstructive and limited insufficient ventilation, which were mainly caused by compression of lung tissue due to increased abdominal pressure, elevated diaphragm and increased chest volume and pressure when patients had additional ascites, and atelectasis [306]. However, decreased FEV1 resulted from compression of small trachea due to pulmonary interstitial edema and vasodilation, and early closure of expiration. Theoretically, all of the above factors can lead to insufficient ventilation, one of the factors resulting in this disease. This was also substantiated by significantly increased arterial partial pressure of oxygen and decreased CO_2 partial pressure in cirrhotic patients with pleural effusion after pleural effusion extraction and recovery from atelectasis.

However, there are also some people who do not think that hypoxemia results from insufficient ventilation, but because cirrhotic patients are not complicated by high concentrations of CO_2 when their arterial partial pressure of oxygen is decreased [311]. This is likely because when patients have hypoxemia, compensation of hyperventilation causes arterial blood CO_2 partial pressure not to increase, and results in decreased $PaCO_2$ or even respiratory alkalosis. Besides, in some patients without decompensated liver cirrhosis, arterial hypoxemia can also occur. Even it has been found that the lung function tests in patients with decompensated liver cirrhosis are normal. Therefore, the majority of scholars currently believe

that insufficient ventilation is not the main cause of hypoxemia in cirrhotic patients.

2.6.6.2 Diffusion Disorder

For patients with HPS, the inert gas exclusion technique should be performed to prove that there is a disorder in the diffusion of oxygen, which is determined by the basic pathological changes of HPS - pulmonary vasodilatation. Pulmonary angiography can show small spider-like to obviously cavernous diffuse vasodilation within the lungs. Due to the significant dilation of the pulmonary capillaries and the anterior capillaries, the diffusion distance of the blood flow in central blood vessels and the alveoli is increased, preventing the gases in the alveoli from entering the pulmonary capillaries, thereby affecting the gas exchanges. Studies have shown that hypoxemia often occurs in patients with cirrhosis or aggravates during exercises, and it is believed that diffusion disorder or limitation of oxygen occurs in patients. In fact, factors that affect O_2 diffusion do occur in patients with cirrhosis, but they are still not sufficient to explain the apparent hypoxemia. Although vascular dilatation occurs at arterial endings in patients with HPS, their arterial partial pressure of oxygen can be reduced while inhaling air and increased when they are given oxygen inhalation, which further proves that although diffusion disorder does exist and it plays a role in the formation of this disease, the role is not important.

2.6.6.3 Ventilation/Blood Flow Ratio Imbalance

To engage in gas exchanges is the most important biological function of lung tissues, and this gas exchange must be completed when there is an appropriate ventilation/blood flow ratio. Under the normal circumstance (normal adult resting state), the most appropriate ventilation/blood flow ratio physiologically is 0.8. Changes in the ratio due to any cause can affect the gas exchange, and the imbalanced ventilation/blood flow ratio in HPS patients with hypoxemia is mainly because of pulmonary vascular dilatation and arteriovenous shunt [312].

1. **Intravascular vascular dilatation:** Pulmonary vascular dilatation has been confirmed pathologically and by angiography. Dilated blood vessels in the lungs leads to gas diffusion disorder. Besides, since the oxygen molecules in the air can not be diffused to the central dilated blood for the gas exchange, causing decreased ventilation/blood flow ratio and pulmonary arterial partial pressure of oxygen. This decreased ventilation/blood flow, together with increased amount of reactive cardiac output, shortens the duration of blood that flows through the capillary network and insufficient oxygenation [312]. Excessive ventilation can in part enhance patients' PaO_2. If the alveolar oxygen partial pressure is increased at this time, some oxygen molecules can reach the central areas of dilated blood vessels, increasing the arterial partial pressure of oxygen. Therefore, it is called the diffusion - perfusion disorder or pulmonary arteriovenous functional shunt rather than the true lung shunt.
2. **Arterial - venous shunt:** Intrapulmonary vascular fistula and pleural spider nevus can occur in cirrhosis of the liver and allow the pulmonary arterial blood

to circumvent gas exchange and directly flow into the pulmonary vein so that patients may develop hypoxemia. This hypoxemia can not be corrected by oxygen inhalation and represents the true pulmonary shunt, which has been confirmed by pulmonary histopathology, angiography, transthoracic echocardiography and other examinations. It is now believed that pulmonary vascular casting is still the most direct evidence for determining the arterial - venous shunt. This intrapulmonary arterial - venous shunt is the main cause of abnormal ventilation/blood flow ratio and insufficient gas exchange. Although pleural spider nevus can also cause arterial - venous shunt, it generally does not suffice to cause significant hypoxemia due to the small amount of shunt. In addition, studies in recent years also show that portal-pulmonary vein shunt in a small amount occurs in some patients with cirrhosis, in whom blood flow circumvents the alveolar gas exchange and enters directly the systemic circulation. This can also cause ventilation/blood flow abnormalities, causing insufficient gas exchange

3. **Airway closure:** In 1971, Ruff et al. proved that cirrhotic patients had significantly increased closed volume (CV) and total amount of closed gas (CC) and increased gases trapped in the lower fields of the lungs, resulting in an extremely low ratio of ventilation/blood flow, and they believed these were due to reduced airway ventilation [302, 313]. In 1984, Furukawa et al. measured the lung function of 105 patients with liver cirrhosis and did not find abnormalities; however, most patients had flow - volume abnormalities and significantly increased CV, suggesting the closed the airway in advance and decreased ratio of ventilation/blood flow, which might important causes of hypoxemia.
4. **Decreased affinity of oxygen with hemoglobin:** Some reports showed that 15 patients with cirrhosis (mostly alcoholic cirrhosis) patients had mild systemic vascular or pulmonary vascular dilatation, normal PaO_2, mild hypocapnia, mild right shift of the oxygenated hemoglobin dissociation curve, normal amount of carbon monoxide diffusion [313], and mild imbalance of the ventilation/PaO_2 blood flow, indicating that the right shift of the oxygen dissociation curve due to decreased affinity of oxygen with hemoglobin in the patients. This was possibly caused by the increased concentration of 2,3-diphosphate glyceride in red blood cells, which, however, is not an important factor in the occurrence of hypoxemia [302].

In summary, hypoxemia can result from many factors, while none of the factors can completely explain the pathogenesis underlying the disease. Since the basic pathological changes in patients with HPS are intrapulmonary vascular dilatation and opening of arterial - venous communicating branches, together with recent findings, it is suggested that the diffusion disorder of the alveoli and pulmonary capillaries and ventilation/blood flow imbalance may coexist, and are the main cause of hypoxemia in this disease. Other factors may aggravate hypoxia and are secondary factors. Therefore, it is believed that the disease occurs as result of the above factors.

2.6.7 Pathological Features

The pathological features of HPS are dilation of capillaries in the anterior aspect of the lungs and telangiectasia. Autopsies show arterial-venous short circuit within the lungs, vasodilation and thickened pulmonary muscles [314]. At the same time, arterial hypoxemia is common in liver diseases, often attributable to a variety of factors (such as ascites, hepatic pleural effusion, and COPD in patients with alcoholism); it shows unique pathophysiological characteristics under specific circumstances of HPS. Its prominent features are dilation of micro-arteries in the anterior aspect of pulmonary capillaries and true capillaries (the normal diameter of these vessels is 8 to 15 μm, which can reach 15–100 μm when patients rest), with the number of dilated vessels increased macroscopically. Some patients show arterial-venous communicating between the pleura and lungs, vascular anastomosis in the liver and the lungs, and thickened walls of small veins and capillaries. Pulmonary vascular dilatation is promoted, and mixed venous blood quickly or directly enter the pulmonary veins through the anastomosis in the lungs, leading to oxygenation defects. Increased nitric oxide (NO) is a key cause for pulmonary vasodilation, and whether other mediators, such as heme oxygenase-derived carbon monoxide, are causes of pulmonary vasodilatation are not yet confirmed. Abnormal arterial oxygenation seriously affects the survival of patients, and is an important indicator that determines the timing and risk of liver transplantation as well as an important basis for the grading of severity of HPS. Causes of deaths associated with HPS are often multifactorial and are associated with basic liver diseases, and there are few cases of respiratory failure due to severe hypoxemia.

2.6.8 Clinical Manifestations

HPS is a triad composed by intrapulmonary vascular dilatation and insufficient arterial oxygenation due to primary liver disease, and it is mainly clinically manifested as primary liver disease and pulmonary lesions.

2.6.8.1 Clinical Manifestations of Primary Liver Disease

HPS can occur in various liver diseases, mostly in chronic liver disease, especially cirrhosis caused by various causes, such as cryptogenic cirrhosis, alcoholic cirrhosis, liver cirrhosis, viral cirrhosis, postnecrotic cirrhosis and biliary liver cirrhosis, etc. The most common clinical manifestations include liver palms [314], spider nevus, jaundice, ascites, hepatosplenomegaly, gastrointestinal bleeding and abnormal liver function, etc., while they are not significantly correlated with HPS. Some patients with clinically stable liver disease may also develop the clinical manifestation of progressive pulmonary insufficiency.

2.6.8.2 Clinical Manifestations of Pulmonary Dysfunction

Since HPS patients have no primary cardiopulmonary diseases, most (80–90%) patients gradually develop respiratory manifestations on the basis of various liver diseases, such as cyanosis, dyspnea, clubbing, orthodeoxidation and platypnea, etc.

Among them, progressive dyspnea is the most common lung symptoms of HPS. Binay et al. believed that cyanosis was the only reliable clinical sign, and that platypnea and orthostatic hypoxia are the most characteristic manifestations. Pulmonary examinations generally show no obvious positive signs [315]. A small number of patients (about 16–20%) can present complaining dyspnea on exertion in the absence of clinical manifestations of a variety of liver diseases, to which attention should be paid clinically so as to prevent misdiagnosis. The domestic researchers Gao Zhi et al. reported that 2 patients presented to hospitals with cyanosis, palpitation after exercises and shortness of breath; meanwhile, they found that the patients had clinical manifestations of liver cirrhosis [308] (such as liver palms, spider nevus, hepatosplenomegaly and ascites), which were conducive to the diagnosis of this disease. If liver disease patients have other lung diseases (such as chronic bronchitis, emphysema and pneumonia, and pleural effusion, etc.), then significant respiratory symptoms may occur. Therefore, differential diagnosis should be made. Data show that the duration of initial dyspnea to the conformed diagnosis in patients with HPS average 2–7 years; that is to say, about 18% of patients already have dyspnea at the time confirmed diagnosis.

1. Orthodeoxidation: PaO_2 is decreased by >10% when patients switch from the supine position to the standing position.
2. Platypnea: When patients switch from the supine position to the standing position, they have palpation, chest tightness and shortness of breath, and when patients resume the supine position, the above symptoms are improved [313]. Krowka reported that about 80–90% of patients with HPS had the above two manifestations because vascular dilatation in the patients was mainly distributed in the middle and low lung fields. When patients switch from the supine position to the standing position, the blood flow in the middle and lower lobes of the lungs is increased under the action of gravity, aggravating hypoxemia [315]. Although the two manifestations are not unique to HPS, they suggest the significant abnormality in the patients' pulmonary vascular system. If patients with a variety of liver diseases present with the above two manifestations, further examinations are needed for confirmation.

2.6.9 Complications

Patients may present with liver palms, hepatosplenomegaly, spider nevus and ascites; patients show palpitation, chest tightness, shortness of breath when switching from the supine position to the standing position due to hypoxemia.

2.6.10 Laboratory and Pathological Examinations

2.6.10.1 Arterial Blood Gas Analysis and Pulmonary Function Tests

Schenk et al. defined the values of PaO_2 for the diagnosis of HPS, thinking that $PaO_2 < 70$ mmHg suggested a high possibility of HPS, and that for $PaO_2 < 65$ mmHg,

the diagnosis of HPS could be made [306]. Pulmonary function tests mainly showed significantly decreased VC, FRC, MVV and FEV1, but sometimes the total lung volume and FEV1 were normal.

Chest X-ray, CT scan and transthoracic contrast echocardiography (TTCE) HPS patients are mostly normal on chest radiography or show diffuse small millet shadows predominantly in both lower lobes of the lungs, nodular shadows in both lower lung interstitium, dilated pulmonary arterial trunks, and thickened pulmonary markings, whereas these manifestations have no specific values to the diagnosis of this disease. CT scan shows certain diagnostic values in that it demonstrates distal vasodilation and even pleural blood vessels, and can suggest the presence of HPS. Arteriovenous communicating occurs in HPS patients due to pulmonary vascular dilation, indicating that subclinical pulmonary vascular dilatation and abnormal gas exchanges occur in cirrhotic patients with normal PaO_2 [306]. Contrast echocardiography: when HPS is suspected, transthoracic echocardiography can be used as a preliminary screening to determine whether the intrapulmonary vascular dilation occurs or not. The microbubble contrast material in the right atrium, after intravenous injection of dioxane isotonic saline, will develop images in the left atrium through the dilated vascular beds after 3–6 cardiac cycles, while the microbubbles cannot pass through the normal capillaries (Normal capillaries are <8–15 μm in diameter). Approximately 40% of patients with cirrhosis had positive changes on contrast echocardiography, while only a small proportion of patients are in line with the diagnosis of HPS due to the influence of dilated blood vessels. If contrast echocardiography is positive for liver cirrhosis or portal hypertension patients with hypoxemia and cardiopulmonary diseases in them can be ruled out, then the diagnosis of HPS is established.

2.6.10.2 Pulmonary Angiography

This means is used to confirm the diagnosis of intrapulmonary vasodilatation. The pulmonary vascular abnormalities in HPS patients are as follows: (1) diffuse spider nevus images. Patients of this type have severe hypoxemia and erectile hypoxia and respond well to inhalation of 100% oxygen; (2) cavernous or spotted arterial dilatation mainly seen in the basal aspect of the lungs. Patients during this period respond poorly to 100% oxygen; and (3) intermittent local arterial malformation or communicating branches, isolated earthworm-like or bulk images. In addition to severe hypoxemia and erect hypoxia, patients of this this type respond extremely poorly to the inhalation of 100% oxygen.

2.6.10.3 ^{99cm}TC-MAA scan

When hypoxemia is caused by HPS and cardiopulmonary diseases concurrently, ^{99cm}TC-MAA scan can determine hypoxemia resulting from HPS more likely. Radiolabeled albumin ^{99cm}TC, which is administered through intravenous injection, is about 20 pm in diameter. When pulmonary vascular shunt occurs, a proportion of the polymerized albumin passes through the lungs and enters the systemic circulation, the intake of albumin by other organs can be simultaneously determined by scintigraphy [308]. Therefore, the amount of shunt can be calculated. A study

showed that $^{99\ cm}$TC-MAA scan was positive for HPS patients with Pa02 < 60 mm Hg, while the scan was negative for chronic obstructive pulmonary disease (COPD) patients with the same degree of hypoxemia, a result indicative of the good specificity of this means. Compared with contrast echocardiography, ^{99cm}TC-MAA scan, in spite of its low sensitivity, can be used for the diagnosis of HPS in patients with COPD.

2.6.10.4 Pathological Examinations

Pathological examinations are the most reliable means for the diagnosis of HPS, whose basic pathological change is pulmonary vasodilation manifested as diffuse anterior capillary dilation or discontinuous formation of arteriovenous branches [312]. In addition, pulmonary perfusion scan and right cardiac catheterization are also valuable for the diagnosis of HPS to a certain extent.

2.6.11 Diagnosis

There is no unified standard for HPS diagnosis to date. Diagnosis should be based on clinical manifestations plus imaging evidence of pulmonary angiography.

1. Rodriguer-Roisin et al. proposed the diagnostic criteria of HPS in 1992:
   (a) Presence of chronic liver disease with or without serious hepatic insufficiency;
   (b) No heart and lung disease, normal chest X-ray examination, or pulmonary basal nodular shadows simultaneously;
   (c) Abnormal lung gas exchange, increase in alveolar - arterial oxygen gradient by (≥ 20 KPa (150 mmHg)), and possibly hypoxemia;
   (d) Existence of pulmonary vasodilatation and/or pulmonary vascular short circuit on contrast-enhanced two-dimensional echocardiography and/or pulmonary perfusion scan, and pulmonary angiography. Such clinical manifestations as orthostatic hypoxia and shortness of breath are important reference indicators.
2. Chang SW et al. proposed the diagnostic criteria for HPS in 1996:
   (a) Hepatic insufficiency.
   (b) Hypoxemia, alveolar gas - arterial oxygen partial pressure difference [P (A-a) O_2] ≥ 2.67 KPa (20 mmHg) when patients are breathing air in the supine position or under orthostatic hypoxia.
   (c) Intrapulmonary vascular dilatation.
3. Krowka et al. believed in 1997 that when portal hypertension, spider nevus and clubbing occurred in patients, the diagnosis of this disease was strongly suggested, and relevant tests were needed for confirmed diagnosis. The diagnostic criteria included:
   (a) Intrapulmonary capillary dilation confirmed by such examinations as ^{99cm}TC-MAA scan, contrast-enhanced transthoracic echocardiography and pulmonary angiography;

(b) Chronic liver disease and hypoxemia with $PaO_2 < 9.3$ KPa (70 mmHg).
(c) The domestic scholars Gao Zhi et al. thought in 1998 that the diagnosis of this disease should be based on the following manifestations in patients, hepatosplenomegaly, ascites, liver palms, spider nevus, dyspnea on exertion, hypoxia while breathing in the supine and orthostatic positions, increased mesenchyma in the basal aspects of the lungs and vascular markings on chest radiography, patchy or nodular shadows, dilated basilar pulmonary vessels and increased pulmonary vascular branches on CT, severe hypoxemia or not on blood gas analysis, increase in alveolar - arterial oxygen gradient by ≥20 KPa (150 mmHg), and 82% diffusion disorder on pulmonary function tests [316]. In addition, shunt-related examinations should be performed, such as ^{99cm}TC-MAA scanning, contrast-enhanced two-dimensional echocardiography and pulmonary angiography, etc., while the last one does not show the same sensitivity as that of the former two because the small blood vessels in the lungs may not develop on angiography.

2.6.11.1 Differential Diagnosis

Most HPS patients have a slow onset of the disease, are difficult to treat, and have a poor long-term prognosis, with a mortality of more than 40% after 3 years. Therefore, early diagnosis of the disease and its differential diagnosis are vital to improving the prognosis of patients.

First of all, the previous liver and lung diseases in the patients should be ruled out, such as chronic obstructive pulmonary emphysema, pulmonary infection, interstitial pneumonia and silicosis, etc. At the same time, cirrhosis with pulmonary hypertension, infections secondary to pleural effusion, interstitial pulmonary edema, atelectasis and hyperventilation syndrome, etc. need to be ruled out. HPS should be differentiated mainly from the following diseases:

1. **Liver cirrhosis following pulmonary heart disease:** This is mainly because pulmonary diseases result in cardiac insufficiency and thus increased pulmonary venous pressure. Repeated or long-term existence of liver congestion can lead to central venous hypertrophy and lobular central connective tissue hyperplasia, and further progression of the lesion will lead to the formation of liver cirrhosis following pulmonary heart disease. Patients with pulmonary cirrhosis often have a long history of chronic lung disease and signs of cardiac insufficiency, such as edema of lower extremities, palpitation, shortness of breath and other symptoms. This patient has no history of chronic lung disease or edema of lower extremities, making him inconsistent with liver cirrhosis following pulmonary heart disease.
2. **Left heart insufficiency:** Both HPS and left heart insufficiency can cause severe dyspnea and hypoxemia. A history of liver disease or evidence of chronic liver damage and decreased PO_2 can be found in patients with HPS, especially such characteristics as orthostatic hypoxemia and intrapulmonary vascular dilatation. Patients with left heart insufficiency have a history of heart disease, orthopnoea,

pink frothy sputum and moist rales in the lungs, etc. This patient is inconsistent with such manifestations.

3. **Primary pulmonary hypertension:** After inhalation of pure oxygen, hypoxemia in most of patients with HPS will be significantly alleviated. The effects of oxygen are poor in patients with hypoxemia [317], and HPS is manifested as orthostatic hypoxemia. The characteristics of hemodynamics of patients with HPS are hyperdynamics and normal or decreased pulmonary artery pressure and pulmonary vascular resistance, while those in hypoxemic patients are increased.
4. **Others:** Ductus arteriosus, Eisenmenger syndrome and pulmonary embolism, etc., need to be differentiated from this disease, and comprehensive judgments should be provided based on other clinical data of the medical history.

HPS patients can also have the above-mentioned diseases, and careful and meticulous examinations are needed for differentiation.

2.6.12 Treatment

Because HPS is developed on the basis of original liver disease, the frequency of its occurrence and its severity are mostly associated with the liver cell function of patients, while there are also HPS patients in whom chronic liver disease is relatively stable and liver functions are normal. Besides, pleural effusion, ascites and infections secondary to pulmonary edema after liver function decompensation can aggravate patients' respiratory function injury. Therefore, under the current circumstances in which there are no effective measures for HPS, active and effective treatment of primary liver diseases is the basis for the treatment of HPS. Therapy of primary diseases, including correction of hypoproteinemia, elimination of pleural effusion, improvement of liver function and treatment of complications, etc., can improve tissue oxygenation and improve arterial oxygen saturation. On this basis, the following treatment can be given.

1. **Oxygen inhalation and hyperbaric oxygen chamber** Oxygen therapy also helps the differential diagnosis of pulmonary shunt: if PaO_2 is resumed after oxygen inhalation, then the diagnosis of intrapulmonary vascular dilatation (IPVD) can be made; for patients with partial improvement, pulmonary anatomical shunt and functional shunt may coexist; for patients in whom the oxygen therapy proves inefficacious, pulmonary arteriovenous fistula is a possible diagnosis. It is now believed that once the diagnosis is established, treatment should be given as soon as possible. In the early stage of correcting hypoxemia in patients with mild conditions, even in patients in whom the critical value of hypoxemia (PaO_2, 8–9 KPa (60–67.5 mmHg) is reached and who have ascites, the hemoglobin saturation may still be less than 85% when patients are in activities or even sleep. That is to say, nasal catheter oxygen inhalation at 2–3 L/min is needed so as to improve hypoxemia [318]. With the development of the disease,

oxygen flow needs to be gradually increased, and intratrachea oxygen supply can be offered when necessary. During the late stage, patients can receive pressurized oxygen through a ventilator or a hyperbaric oxygen chamber. For patients whose conditions are severe, the efficacy of oxygen therapy alone is not obvious.

2. **Vasoactive drugs** Vasoactive drugs for the treatment of patients with HPS are most studied; however, since its pathogenesis has not been clarified to date and primary liver disease is difficult to reverse, it is hard to define the clinical efficacy of these drugs. The commonly used drugs include:
   (a) Almitrine (Vectarion): At first, Krowka et al. clinically used the drug because they believed that the ratio of pulmonary ventilation/blood flow could be changed by increasing pulmonary vascular tension. The results were that the hypoxia symptoms were improved in only 1 HPS patient, and that PaO_2 was increased by >1.33 KPa (10 mmHg). The remaining four patients showed no definite effects. However, the application of this drug in animal experiments and patients with chronic obstructive pulmonary disease could promote the ventilation/blood flow ratio.
   (b) Somatostatin and its analogues: These drugs can block the dilatory effects of nerve peptides on pulmonary blood vessels and suppress the production of glucagon. Salem et al. reported a case in which octreotide could improve PaO_2 swiftly in the patient with liver cirrhosis and severe hypoxemia, and he successfully underwent liver transplantation. However, the study by Krowka and Schwarty et al. showed that this class of drugs did not yield significant effects on patients with HPS. Theoretically, this drug can block the dilatory effects of nerve peptides on pulmonary blood vessels, while there are also data showing that octreotide can stop the symptoms of cancer-like metastatic syndrome. Therefore, its clinical efficacy still merits further studies. Song et al. found that long-acting aspirin displayed a certain effect.
   (c) Prostaglandin inhibitors: They can inhibit the synthesis of prostaglandin E2a within the lungs, reduce the dilatory effects of prostaglandin E2a on pulmonary vascular beds and improve the arterial oxygenation in animals with lung injury. Shijo et al. used indomethacin for the treatment of patients with HPS, and increased PaO_2 and decreased alveolar - arterial oxygen pressure were noted. Still, further clinical applications are merited for confirmation [308].
   (d) Cyclophosphamide and glucocorticoids: Cadranel et al. reported that cyclophosphamide and prednisone for 12 months successfully resulted in improved hypoxemia in a non-cirrhotic hepatocellular failure patient. Maybe these drugs are effective for pulmonary lesions in patients with chronic liver disease due to immune dysfunction.
   (e) Aerosol inhalation of ephedrine: Domestic researcher Zhang Liming et al. used aerosolized ephedrine hydrochloride for the treatment of 12 patients with HPS, and the preliminary efficacy was significant. The mechanisms were that ephedrine could excite the pulmonary vascular α receptor, resulting in contracted bronchial mucosa and pulmonary capillaries and alleviated

bronchial edema, so that the dilated blood vessels within the lungs were contracted and intrapulmonary shunt was reduced. Meanwhile, the bronchial β2 receptors were excited and the bronchi were dilated so as to improve the ventilation/blood flow ratio and relieve hypoxia. Further studies are merited.

(f) Others: There have been reports on sympathomimetic drugs (isoproterenol) and β-blockers (propranolol), etc. that improve the symptoms of HPS. Theoretically, vascular endothelin, estrogen suppressor (Tamoxifen) and so on can relieve the spider nevus and pulmonary vascular dilatation in patients with liver cirrhosis and improve their respiratory symptoms, while further studies are needed. NO is most studied currently, and there are reports indicating that NO synthesis inhibitors can increase pulmonary vascular resistance. Alexander et al. used NO for the treatment of severe hypoxemia in patients after liver transplantation, and obtained good results. Durand et al. also reported that an HPS patient was cured by inhaling NO, while its mechanisms and clinical efficacy needed to be further confirmed.

3. **Pulmonary embolism** It is generally considered that pulmonary vascular dilatation can vanish after liver transplantation in HPS patients who are normal on pulmonary angiography or who have cavernous vessels on imaging [310]; for patients who show diffuse pulmonary vascular dilation features on pulmonary angiography, embolization is usually not adopted since patients' lesions are extensive and the efficacy is poor; for patients with isolated and severe pulmonary vascular dilation or arterial-venous communicating branches, local pulmonary embolism therapy can yield a satisfactory effect.
4. Liver transplantation It is currently considered that liver transplantation is still a possible fundamental measure for the treatment for HPS. In the past, it was believed that serious hypoxemia was an absolute contraindication against liver transplantation, while recent studies show that liver transplantation is preferred for patients who have good alveolar gas diffusion function, who can respond well to pure oxygen inhalation and who can undergone oxygenation safely during anesthesia. Recent reports further prove that hypoxemia can be cured after liver transplantation [308]. Through literature review and case reports, Krowka et al. believed that progressive hypoxemia in HPS could be used as an indication of liver transplantation. Temporary hypoxemia following liver transplantation can be adjusted by using NO and taking the head-down supine position and the alternate lateral decubitus position. And for HPS patients who fail to respond to the inhalation of pure oxygen, who have direct pulmonary arterial communicating branches on pulmonary angiography and who have severe clinical hypoxia, liver transplantation cannot improve their hypoxic status, has limited efficacy, or even increases intraoperative and postoperative risks. Therefore, liver transplantation should not be performed on them.
5. Transjugular intrahepatic portosystemic shunt (TIPS) Selim et al. believed that TIPS was an effective method for the treatment of HPS, and its effects of improving symptoms, enhancing oxygenation and reducing intrapulmonary shunt could last up to 4 months. Riegler et al. performed TIPS on an HPS patient with diffuse

intravascular dilatation who was not suitable for vascular embolization, and the results showed significantly increased PaO_2 and significantly improved hypoxemia. However, Coley et al. also reported that a patient failed to respond to TIPs, and therefore, its exact effects remain to be studied.

6. Other treatment options One patient with HPS was once treated with garlic, and 18 months later, his oxygenation was significantly improved and his symptoms were relieved. There are also patients who receive plasma replacement therapy, which has limited effects on the oxygenation of patients with HPS [308].

To sum up, no effective treatment options are currently available for HPS. Since the basic cause of HPS is liver cell failure, the usual cause of patients' deaths is not lung failure, mostly complications such as gastrointestinal bleeding, renal failure, hepatic encephalopathy and sepsis. Therefore, we consider that the therapy of primary liver disease is particularly important. Oxygen inhalation alone can be given in the early stage of hypoxemia, or conservative treatment can be provided if additional drugs are effective. Liver transplantation is the best solution whenever possible. It is generally accepted that liver transplantation is the most promising regimen with confirmed efficacy. If oxygen inhalation is less satisfactory and patients are diagnosed with local intrapulmonary vascular dilatation or arterial - venous fistula by such means as pulmonary angiography, pulmonary embolism should be carried out as soon as possible. For patients with additional obvious portal hypertension, TIPS treatment can also be given.

2.6.13 Prognosis

The interval from chronic liver disease and cirrhosis in patients to the confirmed HPS due to such respiratory symptoms as anoxic dyspnea is usually several years or even more than 10 years [average interval, (4.8 ± 2.5) years], and a small number of patients can develop such a disease acutely in the short term. Besides, signs of chronic liver disease can be traced in patients complaining breathing difficulties. Once HPS is established, obvious hypoxemia has occurred already. It confers a poor prognosis, and most patients die within 2–3 years often due to other complications of liver disease [312, 315]. If patients' oxygenation is satisfactory and they have undergone liver transplantation, or with the improvement in liver function, their hypoxemia can be resolved or improved of its own volition with good prognosis. If patients' oxygenation deteriorates severely and they have a very poor prognosis, most of them will die in the short term.

HPS often progresses slowly. Although it is not a direct cause of death in patients with cirrhosis, it can significantly aggravate the disease. Therefore, cirrhotic patients, especially those with positive liver palms and spider nevus as well as patients with portal hypertension, should be careful of the possibility of HPS. Timely detection and symptomatic treatment (such as low flow oxygen inhalation) can improve the prognosis of patients.

2.6.14 Prevention

Active and effective treatment of primary liver disease forms the basis for the prevention of this disease. Education of common sense should be given to patients with liver diseases so as to avoid factors inducing HPS in their life. For patients with liver disease, mild HPS should be found as soon as possible and appropriate treatment should be given.

2.7 Section 7: Other Complications

Jia-Quan Huang and Dong Xu

2.7.1 Endotoxemia

2.7.1.1 Metabolism and Biological Activities of Lipopolysaccharide

Lipopolysaccharide (LPS) is a constituent of bacteria cell wall which plays an essential role in the pathogenesis of septic shock by generating endogenous mediators such as nitrous oxide, cytokines, superoxide anions, and lipid mediators. Despite the recent advances in antibiotic treatment and hemodynamic monitoring, septic shock still remains a serious disorder that is associated with a high mortality rate, to more comprehensive definition of the mechanisms that underlie innate immunity against bacterial pathogens, LPS has been extensively studied [319]. The pathophysiological consequences of bacterial sepsis are contributed by the dysregulation of these same mechanisms. Before we can hope to design effective anti-sepsis therapies, greater insight into the nature of host interactions with LPS is extremely essential.

The Structure of the Lipopolysaccharide

The Gram-negative bacterial envelope is composed of two bacterial membranes, outer and inner membrane. The outer membrane consists of the following substances, like lipopolysaccharide (LPS), several kinds of outer membrane proteins, lipid A and metal ions. For most Gram-negative bacteria, LPS is a major component in the outer monolayer of the outer membrane which works like a tight shield. The shield is composed by unique molecules, such as polysaccharide, or long chain of sugar, and lipid A. During the process to evoke the signaling events of LPS, lipid A plays a pivotal role. The entire lipid component of LPS molecule, however, is required for optimal activity [320].

Biological Activities of Lipopolysaccharide

The basic principles of LPS bioactivity are nowadays well understood. Endotoxins do not elicit their toxic effects - as we might suspect and as it is known for many proteinous exotoxins which can kill host cells or inhibit cellular functions. Rather, LPS requires the active response of host cells. According to present knowledge we

get, LPS interacts with various host cell types through lipid A, those cells include mononuclear cells, thrombocytes, endothelial and smooth muscle cells, and polymorphonuclear granulocytes, among which macrophages/monocytes are of particular importance. Through the LPS-induced activation, macrophages produce many substances, like bioactive lipids, reactive oxygen species, and in particular, cytokines such as tumor necrosis factor a (TNF), interleukin-1, IL-6, IL-8, and IL-10. It appears that when low levels of mediators are produced, beneficial effects (e.g., induction of resistance to infection, adjuvant activity) are elicited and when high levels of mediators reach the circulation that detrimental effects (e.g., high fever, hypotension, irreversible shock) are induced. However, when the host organism is in a hyperreactive state LPS, low mediator concentrations may also become harmful. The hyperreactivity to endotoxin may be caused by exo-toxins, chronic infection, and by growing tumors, and interferon-γ.

Endotoxemia Metabolism and Host-Defence

To function properly, organism requires an immune system that must detect pathogens, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. The immune system can be classified into humoral immunity versus cell-mediated immunity or the innate immune system versus the adaptive immune system.

When microbes invade organism, the innate response is usually triggered by pattern recognition receptors, which recognize components that are conserved among broad groups of microorganisms, or when injured, damaged, or stressed cells send out alarm signals, many of which (but not all) are recognized by the same receptors as those that recognize pathogens. The innate immune system defenses are nonspecific, meaning that the system responds to pathogens in a generic way. This system does not confer long-lasting immunity against a pathogen. In most organisms, the innate immune system is the dominant system of host defense [321].

They activate innate immune responses by identifying some conserved non-self molecules, so as to protect the host from infection,. Bacterial lipopolysaccharide (LPS), an endotoxin which is found on the bacterial cell membrane, is considered to be the prototypical PAMP. LPS is specifically recognized by Toll-like receptor (TLR) 4, a recognition receptor of the innate immune system.

The interaction of the lipid A moiety of LPS with macrophages appears to be especially important because subsequent cellular activation results in the release of systemically active pro-inflammatory molecules, which in turn mediate systemic toxicity. LPS has extreme potential in macrophages activated at concentrations of LPS as low as 1 pg/mL [322]. Host-defense peptides (HDPs) could be a possible alternative solution since they possess the antimicrobial, antiseptic, and immunomodulatory properties [323].

The Release of Endotoxin

Endotoxins lipopolysaccharide is released not only from dead Gram-negative bacterial, but also from the growing ones. Endotoxins are very stable molecules, which are resisted to extreme temperatures and pH values in comparison to proteins. Endotoxins are shed largely during cell death as well as growth and division. They

are highly heat-stable and are not destroyed under regular sterilizing conditions. Endotoxin can be inactivated through exposed at temperature of 250 ° C for more than 30 min or 180 ° C for more than 3 h. Acids or alkalis of at least 0.1 M strength can also be used to destroy endotoxin in laboratory scale [324].

Endotoxin Translocation

Gut microbiota is composed of strict anaerobes, facultative anaerobes and aerobes. Recent reports suggest the existence of over 35,000 bacterial species in the human gut microbiota. An important characteristic of gut microbes is their heterogeneity [325].

The composition and the frequency of the microbiome changes with the different segments of the elementary tract. The composition is influenced by the environment, consumed diet and host factors. Endotoxin to surrounding tissues and organs or blood shift that shift pathway include: (1) via the portal vein, liver into the systemic circulation; (2) through the intestinal tract into the lymphatic system lymphatic; (3) through the intestinal wall into the peritoneal cavity and then absorbed into the bloodstream.

Endotoxin Removal and Detoxification

Under physiological conditions, although a small amount of toxins continued to parenteral shifted via the portal vein into the liver, but it does not cause endotoxemia; mild in Gram-negative bacilli infections, although bacteria continue to release to the tissue or blood endotoxin, but it does not give rise to a strong inflammatory response, the above are dependent on the presence of an effective mechanism within the body to remove toxins and detoxification. The liver is the main organ of clearance of endotoxin, and the spleen, also removes toxins. Molecules in LPS removed include cationic antimicrobial peptides (cationic antimicrobial peptides, CAP), acyloxy acyl hydrolase (acyloxyacyl hydrolase, AOAH) lipoprotein binding protein and anti-LPS antibodies are important endotoxin clearance methods [326, 327].

Liver

Liver blood endotoxin clearance, primarily through Kupffer cells, hepatocytes internal toxin endocytosis achieved, but the specific metabolic process is not entirely clear. Mediated by Kupffer cells engulf toxins may be scavenger receptor, it may be a molecular weight of 119,000 and 83,000 protein; mediate phagocytosis liver toxin structure may be the lectin-like receptor (lectin-like receptor). Endotoxin receptor hepatocyte sinusoidal plasma membrane on the surface: After one to one binding, is taken up within the liver cells endocytosis way to microtubule-dependent vesicular transport through the liver cells, transported to the liver cells bile duct surface to exocytosis into the bile duct, and then discharged into the biliary system through the intestine [328].

Spleen

Splenic macrophages containing approximately 15% of the body to settle within the organization, and macrophages are important endotoxin removal cells. When endotoxin intravenously into the body, except gathering in the liver, a lot of endotoxin

can be quickly gathered and taken up into macrophages in the spleen, the spleen and therefore equally important Endotoxin Removal organs. In addition to its clear role in the performance of its direct effect, but more importantly, spleen macrophages is the precursor cells of Kupffer cells in the liver, having a very big impact on removing toxins within the liver [329].

Intestinal

Mechanisms for removing toxins in the intestine are related to the intestinal villus tip epithelial cells. Under normal circumstances, injected into the intestinal, endotoxin in intestine does not enter intestinal epithelial cells, but after intravenous injection of endotoxin, endotoxin may enter intestinal epithelial cells inside. Therefore, endotoxin receptor may identify ways by endotoxin and (or) simple diffusion way into the intestinal epithelial cells. Endotoxin way into the intestinal epithelial cells intravenously may have two: (1) displaced from the lamina propria macrophages to intestinal epithelial cells basolateral; and enter intestinal epithelial cells from the side; (2) including lower toxin, intestinal lamina propria macrophages, intestinal epithelial cells release large amounts of NO and oxygen free, resulting in intestinal lamina propria microvascular injury, increased permeability, extravasation of endotoxin and ultimately displaced into the outer intestinal epithelial cells. Villus tip epithelial cells within a stronger uptake of toxins, thus endotoxin can be started from the crypt, moving along the intestinal villi, and finally to the top of the inner hair cells of the intestinal epithelium. Uptake of endotoxin villus tip epithelial cell loss, while the endotoxin into the intestine, which constitutes one of the effective clearance mechanisms of endotoxin [327, 330].

Plasma Lipoprotein

Plasma lipoproteins in endotoxin detoxification mechanisms play an important role, in which the lipopolysaccharide binding protein (lipopolysaccharide binding protein, LBP) and high-density lipoprotein (high density lipoprotein, HDL) play a major role. Endotoxin into the bloodstream within minutes there were half white blood circulation due to binding to the edge of the pool or the pool is cleared, the remaining residual endotoxin and rapidly bound to plasma lipoprotein is inactivated. In plasma, lipoproteins and endotoxin when HDL plays a major role in its binding of endotoxin to reach more than 50% of the total, HDL, and thus research endotoxin important [331].

A cationic Antimicrobial Peptides

Cationic antimicrobial peptides are an ancient ingredients in the natural evolution of the immune system, including bactericidal/permeability-increasing protein (bactericidal/permeability-increasing protein, BPI), Cathelicidin, lactoferrin, defensins and other substances, with not only the activity against gram-negative bacteria, but also the ability to combine internal toxins. Cationic antimicrobial peptides are mainly in regular contact with the pathogen site mammalian skin, digestive tract, respiratory tract, and inherently express or express induced by pathogens and their products in the blood, secretions and neutrophil granules. Cationic antimicrobial

peptides have two types of three-dimensional structure; one is a α- helix, having such a molecular structure include Cathelicidin and lactoferrin; the other is β- fold, including mammals α and β-defensins, etc. [332]

AOAH

AOAH is a glycoprotein produced by white blood cells with weight of 5.2–60,000, the large subunit of 50,000 and small subunits of 1.4 million to 20,000, the large and small subunits connected by covalent disulfide bond. AOAH as a lipase with hydrolysis for toxin, can selectively hydrolyze the secondary acyl chain on lipid A acyl groups acyloxy. When hydrolyzing endotoxins, both the large and small subunits of AOAH play an important role, and both are indispensable. In addition to directly destroying toxin, the deacylated LPS after the hydrolysis of endotoxin by AOAH is also involved in AOAH's detoxification mechanism on endotoxin; the material can accumulate and inhibit endotoxin-induced inflammatory response in the cell. However, due to a limited number of secretion by local infiltration of leukocytes, the internal detoxification of toxins is also limited [333].

Anti-toxin Antibodies

When the body respond with endotoxin, one trigger inflammation, on the other hand can be cleared to produce or activate, specific polysaccharide inactivate toxins, including antimicrobial-specific polysaccharide antibody and anti-core polysaccharide antibody. After two antibodies binding with endotoxin, and then with the Fc receptors on the cell membrane, inner source of the toxin-mediated, so that the endotoxin inactivated intracellularly. Anti-endotoxin antibodies can interfere with toxin within LBP binding, thus preventing LBP endotoxin transport [334].

Inhibition of the Body's Defense System

In the body's defense system, the shift from the inhibition of intestinal endotoxin ingredients include intestinal mucosal mechanical barrier, intestinal mucosal immune barrier, the normal intestinal flora and liver hepatocytes and Kupffer cells, in which intestinal mucosal mechanical barrier, intestinal mucosal immune barrier, hepatocytes and Kupffer cells play a direct inhibitory effect, while the normal flora plays an indirect inhibition. The intestine is huge "endotoxin Library", a special anatomical location determines the intestinal mucosa must be an effective defense barrier. Immunological barrier intestinal barrier formed by the epithelial cells of mechanical barrier and secretory IgA (sIgA) and the like components [335].

Intestinal Mucosal Mechanical Barrier

Intestinal epithelial cells and tight junction formation mucosal mechanical barrier, is a significant barrier in the intestine endotoxin translocation defense to maintain its integrity is it to play an important role in defense guarantee. In severe trauma, burns, infection, considerable loss of body fluids, hypovolemia, cause the body ischemia and hypoxia. In order to maintain blood pressure, to ensure that the blood supply to the heart brain and other vital organs, compensatory splanchnic vascular contraction, including gastrointestinal ischemia and hypoxia longer time than other

organs, even after shock patients after resuscitation to restore normal hemodynamics, stomach intestinal still in a state of shock occult. Therefore, when the intestinal microvascular perfusion recovery, intestinal ischemic/reperfusion injury, epithelial cells produce large amounts of reactive oxygen species and other media, resulting in intestinal epithelial cell apoptosis, destruction of tight junctions between cells, thus rapidly increasing intestinal permeability mechanical barrier function weakens, thus contributing to the intestine of the endotoxin absorbed through the intestinal wall to parenteral tissue displacement [336].

Intestinal Mucosal Immunology Barrier

Intestinal mucosal intestinal immunology barrier is a defense of the invasion of pathogens and endotoxin important line of defense, sIgA plays an important role in intestinal mucosal immunity. sIgA is an important component of the protection of the intestinal mucosa, both to prevent bacteria in the intestine mucosal surface colonization, but also in endotoxin. Studies have found that E. coli O157 infection suffered intestinal mucosa, the anti-endotoxin core polysaccharide-specific sIgA secretion, in convalescent patients has been particularly evident, suggesting sIgA endotoxin to prevent the transfer of the body has a protective effect.

In addition, studies suggest that nitric oxide (nitrogen monoxide, NO) preventing endotoxin translocation in intestinal mucosal barrier oxide formed locally. Under physiological conditions, nitric oxide synthase (inducible nitric synthase, iNOS) expression only in the respiratory epithelium, the pregnant uterus and ileal mucosa and a few other parts. Induced by endotoxin including but under normal colonic epithelial cells also express iNOS and catalytic synthesis of NO, are formed in the local oxidation barrier to prevent bacterial translocation colon, thus effectively preventing bacterial translocation, also indirectly prevents endotoxin shift [337].

Bacteriological Barrier from the Formation of the Normal Intestinal Flora

Under physiological conditions, intestinal flora forms a relatively balanced micro-ecosystem. Flora Distribution in the intestine has certain rules: deep close to the intestinal mucosal surface, parasitic anaerobic bifidobacteria and lactobacilli, these anaerobic bacteria are sugar coated, relatively stable, known as membrane flora; middle class bacteria, Streptococcus digest, Veillonella and excellent bacilli; the surface of E. coli and enterococci, can swim in the intestine, known as cavity flora. The antagonism between the layers flora, mutual cooperation, to maintain a dynamic equilibrium, in which the film anaerobic flora is a very important body's natural defense barrier that can prevent opportunistic pathogens such as E. coli colonization in the mucosa, but also can inhibit the overgrowth of opportunistic pathogens.

Intestinal flora micro-ecosystem is a very sensitive system, in severe stress or long-term systemic administration of large doses of broad-spectrum antibiotics, etc., the film significantly reduced the number of anaerobic bacteria, E. coli and other bacteria thrive conditions and continuous release of endotoxin to the intestine, since the film flora defense decreased, these opportunistic pathogens to colonize the intestinal mucosa, resulting in intestinal mucosal barrier damage, followed by the occurrence of intestinal bacteria, endotoxin translocation [338].

Liver Scavenging

Under normal circumstances, the liver is one of the major barriers preventing endotoxin translocation, via the portal vein into the liver hepatocytes and a small amount of the toxin can be Kupffer cell depletion. In conditions such as stress, not only liver cell dysfunction, so the ability to reduce endotoxin detoxification and collaterals between the portal vein and the vena cava, causing an overflow of endotoxin from the liver into the systemic circulation. Endotoxin absorbed into the bloodstream, which in turn may increase the intestinal epithelial cells of the intestinal microvascular endothelial cell damage and, in a vicious cycle [339].

Several Important Cell Endotoxin Recognition

When the body's defense system to produce responses in endotoxin, the innate immune system plays a major role. Pathogens can be identified conserved receptors called pattern recognition molecules (pathogen-associated molecular patterns, PAMPs), including endotoxins of gram-negative bacilli, peptidoglycan Gram-positive cocci, LTA and other cell wall composition and Gram-negative bacteria, Gram-positive bacteria such as DNA. Although a variety of pattern recognition molecules of different chemical structures, but they have similar characteristics: (1) characteristic structure in which different types of pathogens in a relatively constant conserved; produced by a pathogen, the host body without these molecules; survival or disease-causing pathogen is generally the essential, such as mutations, death or loss of a pathogen will pathogenicity. Natural immune system to recognize the receptor molecule called pattern recognition receptors (pattern-recognition receptors, PRRs), including CD14, Toll-like receptor family (Toll-like receptor, TLRs) and scavenger receptors. But in recognition of toxins, some differences exist between the different kinds of cells [340].

Macrophages

Macrophages in addition to expressing CD14, TLRs and scavenger receptors and other associated endotoxin receptors on the cell surface, but in the cytoplasm also express the protein molecules Nod1 recognizing toxins. CD14, TLRs are key receptors that mediate endotoxin within macrophage activation; and scavenger receptor has relationship with macrophage clearing and inactivating toxins [341].

Kupffer Cells

Kupffer cell is the main cell that clears the endotoxin in the liver. Under physiological conditions, although there is still a small amount of bacteria and endotoxin via the portal vein into the liver, but Kupffer cells will clear. Kupffer cells are the most resident macrophages in the liver and are the largest number of resident cells in tissues. There is a theoretical speculation that if Kupffer cells and on is very sensitive to endotoxin as other macrophages, the cell will be in constant activation, but in fact when Kupffer cell engulfs, removes endotoxin, its itself is not activated by endotoxin, which suggests that in the treatment of endotoxin, Kupffer cells have different mechanisms with other macrophages: Kupffer cells treats endotoxin mainly depending on its phagocytosis. In the absence of serum, the phagocytic effects of Kupffer

cell on endotoxin can play a normal; and with the appropriate increase in endotoxin concentration, the phagocytic activity of Kupffer cell was enhanced. The effect has something to do with phosphorylation events of two protein tyrosine residue individually weighting 11.8 million and 8.3 million. CD14 is the main receptor that mediates endotoxin activating Kupffer cell, scavenger receptor is Kupffer cells' important defense of receptor mediated Kupffer cell to remove and inactivate endotoxin.

There are four stages of the activation of Kupffer cells, of which CD14 is the characteristic marker of cellular activation and function change. (1) The stationary phase; the performance of less number of Kupffer cells, small shape, a number in the hepatic sinusoids, CD14 staining negative; (2) reaction period: for the local Kupffer cells stimulate hyperplasia and systemic mononuclear macrophage intrahepatic accumulation; (3) pre excitation period: Kupffer cell phenotype occurred transformation period, expressed CD14 cell membrane receptor, Kupffer cell functional changes; (4) the activation period: the performance of nuclear transcription factor NF KB activation, cellular secretion cytokines [342].

Neutrophils

The CD14 (CD14 membrane-bound, mCD14) and TLR4 endotoxin were activated by the neutrophil surface to activate the neutrophils by binding with the receptor. In addition to the expression of the high affinity endotoxin receptor CD14, the surface of the neutrophils also expressed the low affinity endotoxin receptor L- and the activated cells were activated by the receptor. In addition, the integrin is considered to be a low affinity endotoxin receptor for the surface of the neutrophils [343].

Endothelial Cells

It is generally believed that the expression of mCD14 was not on the surface of endothelial cells and serum soluble CD14 (soluble CD14 (sCD14) is mediated endothelial cell recognition of LPS molecules, and TLR4 is involved in LPS induced endothelial cell activation. LBP was transported to sCD14 by endotoxin, and TLR4 was activated by LPS/sCD14 and activated endothelial cells in the endothelial cell membrane. SCD14 in addition to mediated endothelial cell activation and also mediated by endothelial removal of endotoxin and LPS/sCD14/LBP form trimers and binding to endothelial cells, following the LPS/sCD14 endogenise, thus the removal of endotoxin [344].

Intestinal Mucosal Epithelial Cells

The epithelial cells of the intestinal mucosa were consistently associated with the bacterial and its products, and the bacteria and its products could stimulate other types of cells and induce inflammatory response, but did not induce intestinal epithelial cell defense, this feature for colonic epithelial cells is particularly important, because if can react to the normal intestinal flora in intestinal epithelial cells, it will cause adverse effects on the body. But this does not mean that intestinal epithelial cells are immune cells, when suffered pathogens and their products invasion, intestinal epithelial cells produce normal response. Description: intestinal epithelial cells

with normal differentiation of natural bacteria and pathogenic ability, and the recognition system of subcellular localization. There are different endotoxin recognition mechanisms in the intestinal epithelium and the myeloid cells [327].

Endotoxin and Uncontrolled Inflammatory Reaction

Uncontrolled inflammatory responses (uncontrolled inflammatory response) has a relationship with infection, bacteremia, septicemia, sepsis, systemic inflammatory response comprehensive sign (systemic inflammatory response syndrome (SIRS), compensatory anti-inflammatory response syndrome (compensatory anti-inflammatory response syndrome, CARS) and other related terms used for a long time, but also has an essential difference. Out of control including inflammatory reaction syndrome (MSAs anti-inflammatory response syndrome, Mars), a dynamic process of SIRS and cars and the mixed antagonistic response syndrome, at present clinical many diseases occurrence and development are closely related. Uncontrolled inflammation is a common pathological phenomenon in clinic, which is the important mechanism of the development of the complication after trauma. LPS is one of the main factors that induce the uncontrolled inflammatory reaction in the most common. LPS receptor on the Monocyte/macrophage surface is the he initial factor for the body to recognize and start inflammatory reaction, also is one of the key links for the induction of uncontrolled inflammatory response.

The concept of uncontrolled inflammatory response refers to inflammatory disorders then resulting in multiple organ dysfunction syndrome (multiple organ dysfunction syndrome. MODS), emphasizes the importance of the of balance inflammatory/anti-inflammatory mechanism in the body, changes the limitations that in the past we only attached importance of the pathogenic effects of inflammatory factors. It is believed that the response of the body to the inflammatory factor is the dominant factor in the development of the whole body inflammatory reaction and MODS. This concept is more focused than the previous focus on the dynamic changes of the whole process of inflammation. This can be divided into two types of MODS: one is the early stage after the injury, that is, the speed hair style. The main blame is a strong inflammatory reaction induced by pro-inflammatory factors, and the other is a late phase of the disease, which is “late style”, mainly due to the immune paralysis or worse immune disorders caused by CARS or MARS.

The inflammatory is actually a kind of medium disease mainly caused by the chain reaction of cytokine. Endotoxin is thought to be one of the most important predisposing factors in the chain reaction and can be referred for chain reaction “trigger” (trigger). Endotoxin induced inflammation mechanism is mainly mediated by PAMPs that can induce cytokines such as IL-1, TNF alpha and other active molecules synthesis, the formation of the cytokine network, has a very important role in the occurrence and development of infection. The excessive activation of cytokines can cause septic shock, and is the leading cause of death in patients with bacterial infections. Accordingly, PRRs plays an important role in innate immunity and inflammation, and it can distinguish the pathogens from self organization through PRRs organism, which is characteristic of immune response [345].

2.7.1.2 Systemic Inflammatory Response Syndrome

Inflammatory response syndrome systemic (SIRS) is a systemic inflammatory response caused by any pathogenic factor to the body. The concept is first proposed by Coris in 1985. 1991 August American College of chest physicians and critical care medicine to present the diagnostic standard of SIRS, think to have the following each of the two or more than two, SIRS can be established: (1) temperature > 38 DEG C or < 36 DEG C; II heart rate 90 beats/min; (3), the breathing frequency > 20 times per minute or arterial blood carbon dioxide into pressure ($PaCO_2$) < 4.27kpa 32 mmHg; (4) peripheral white blood cell count >12 × 109/L or 4 × 109/L < or immature myeloid cells >10%. What should be paid attention to is that SIRS is a common athophysiological state of body with severe inflammatory reactions, and should be differentiated from some abnormal factors such as leukemia or cause increase or reduction of white cells after chemotherapy. Although the naming of SIRS has been generally concerned, but some scholars have raised objection to the concept, for example SIRS has following problems: the sensitivity and specificity of the diagnostic criteria is poor, has the same meaning with the "critical"; can not understand the pathophysiology of the original disease; is difficult to guide clinical trials and practice.

The production of SIRS can be divided into two cases, the SIRS caused by the infection and the non infectious SIRS. From the point of view of the clinical development process, SIRS can be followed by injury immediately aroused, then known as the "single phase velocity hairstyle; also to start local, and later developed into a systemic Sirs, namely after the initial shock is brief period of stability, later gradually intensified when SIRS is called" dual phase delayed onset. Either of the factors or the clinical development process, the systemic inflammation of the control of the uncontrolled, and ultimately can lead to MODS [346].

2.7.1.3 The Relationship Between Endotoxemia and Severe Hepatitis B

Endotoxemia

The intestine is the biggest bacterial and endotoxin warehouse in the body. In severe trauma, systemic infection, intestinal ischemia and liver disease, there may be the occurrence of endotoxin. The main source is due to intestinal Gram-negative bacteria in the excessive growth and reproduction, or due to increased intestinal permeability LPS entry into the portal vein increased. If hepatic Kupffer cell phagocytic function is low, the amount of endotoxin over the liver ability to remove endotoxin can "flood" (spill over) into the body of the loop and the endotoxemia formated. Because of the endotoxin from the gut, so it is called intestinal endotoxemia (intestinal endotoxemia, IETM) [347].

The Formation Mechanism of Viral Hepatitis Complicated By IETM

Hepatitis B patients are often accompanied with IETM. Its formation mechanism is: the production and absorption of intestinal endotoxin increased. There is a large number of gram negative bacteria in the body's normal intestinal, so endotoxin in

intestinal contents is very high, but the intestinal mucosal epithelial cells have stronger resistance to toxins so that endotoxin is not easy to run through the intestinal mucosa into the blood, even a small amount of endotoxin breaking through the intestinal mucosal barrier into the portal vein, will be swallowed up by the Kupffer cells in the liver. Severe hepatitis B when the intestinal flora disturbance, endotoxin increased, increased intestinal hyperemia, edema and the permeability of the intestinal mucosa; endotoxin itself can damage the mitochondria and lysosome of intestinal epithelial cells, leading to epithelial cell autolysis; endotoxin can cause intestinal microvascular contraction of the intestinal mucosa, reduce blood, intestinal ischemia, hypoxia, cause the intestinal mucosal barrier function decreased, increased the absorption of endotoxin; severe hepatitis, due to intrahepatic bile acid and bilirubin deposition in Kupffer cell phagocytosis was inhibited, resulting in the removal of endotoxin in the endotoxin decreased; through the door body circulation circuit into the systemic circulation, resulting in blood within liver cells to escape Kupffer toxin the phagocytosis and clearance, which aggravate endotoxemia; the endotoxin can also pass through the celiac lymphatic system into systemic circulation by thoracic duct. In addition, severe hepatitis patients with sepsis, spontaneous bacterial peritonitis, etc., in the release of endotoxin, so the formation of the endotoxin is exogenous [348].

IETM and Severe Hepatitis

In viral hepatitis and other basic diseases complicated with IETM and liver function failure are closely related and endotoxin can directly cause arterial vasoconstriction, the organ ischemia; endotoxin can activating endogenous clotting system, coupled with Kupffer cell dysfunction, decrease delimination of blood coagulation or fiber soluble substances, easily lead to DIC, so as to damage to multiple organs. Endotoxin activated phospholipase A2 mediated membrane phospholipid degradation and lipid peroxidation, which is an important part of liver cell damage. Nolan has pointed out that the effect of Kupffer cell dysfunction induced by intestinal endotoxemia on liver and body, far more than the direct action the endotoxin, and production and release of inflammatory mediators and factors from Kupffer cells activated by endotoxin are closely related. The occurrence of IETM affect hepatic energy metabolism, resulting in liver cell damage and necrosis, also caused hepatic microcirculatory disturbance, performing liver hemorrhagic necrosis. On the basis of severe hepatitis, it can accelerate liver function failure [349].

The Clinical Characteristics of Severe Hepatitis Complicated with Endotoxin

Hepatic Cellular Jaundice

The acute and chronic liver function is often accompanied by intrahepatic cholestasis jaundice, and IETM plays an important role in the occurrence of intrahepatic cholestasis.. Endotoxin involves in liver cell damage mainly through activation of phospholipase A2, and inhibits the activity of Na + − K + − ATPase on liver canalicular membrane to make the bile excretion disorder, and then to cause intrahepatic

cholestasis in the liver cells. Endotoxin can start the peroxidation of liver parenchymal cells mitochondrial membrane lipid so that an increase in the content of oxygen free radical in blood, resulting in the disorder of energy generation, ATP was reduced, so that the active uptake, metabolism and secretion of bile acid by liver parenchymal are short of energy, resulting in cholestasis [350].

Bleeding

The liver disease with IETM often causes the coagulation dysfunction, the serious person appears the different degree bleeding, in particular the severe liver disease patient may concurrent DIC, endangers the life. Violi found that, when liver dysfunction in patients with IETM, the expression of tissue factors on the surface of macrophages and endothelial cell factor induced by endotoxin increased, promoting the synthesis of tumor necrosis factor (tumor necrosis, factor, TNF) to increase, and thrombin generation increasing, activation of coagulation system in about 70% of patients, followed by hyperfibrinolysis, suggesting that IETM in liver dysfunction can be used as the warning signal as the activation of coagulation and fibrinolysis system and activate, and with the increased hepatic lesions, plasminogen activation decreases with endotoxin levels increased, thus plasminogen may decline with endotoxin induced by chronic consumption of DIC on the microstructure of II related factors, new blood can not correct. In fact, before variceal rupture bleeding, patients with liver function severely damaged already have the gastrointestinal mucosa extensive ischemia and erosion, which is the potential causes of bleeding, IETM in the process. And gastric H + at this time can occur abnormal reverse diffusion and stimulate mast cells to release histamine, may lead to mucosal blood vessel dilation and permeability enhanced. As a result, hemorrhage, edema; histamine and directly stimulate the secretion of gastric acid, so that an increase in the number of H + and reverse diffusion, lesions persisted, form a vicious circle [351].

Hepatic Ascites

It is important for the formation of ascites in ascites due to the obstruction of the hepatic vascular outflow tract obstruction. The initial vascular response to endotoxin was the rapid obstruction of the hepatic venous outflow tract and increased the portal pressure which may be related to endotoxin induced swelling of Kupffer cells, liver cells with microvilli swelling, platelet aggregation and fibrin deposition effect, while others think that is endotoxin of blood vessels of the liver has a direct effect. IETM continuous damage to the liver cells, resulting in albumin synthesis and of hormones such as aldosterone de live function obstacles, thus affecting the renal function, and led to the emergence of the refractory ascites plays an important role in the process [352].

Renal Damage

Patients with severe liver disease always are complicated with the functional renal failure, hepatorenal syndrome (HRS). The patients with severe liver disease can be associated with the pre-renal azotemia and acute renal tubular necrosis, and there is a certain relationship with IETM. The clinical studies showed that the levels of

NO_3- - NO_2 and endotoxin in serum of patients with liver cirrhosis were significantly higher. At the same time, plasma renin activity, aldosterone and vasopressin levels increased and urinary sodium excretion decreased. The mechanism about IETM induced by HRS is not clear, may be related to the following factors: leukotrienes (leukotrienes, LTs) LTs can lead to renal vasoconstriction, increased renal vascular resistance, reduced renal blood flow and renal blood redistribution, decreased glomerular filtration rate induced by HRS, in IETM LTs generation and release increased obviously. In addition, liver dysfunction, liver's uptake, inactivation and excretory function of LTs decline, causing blood concentration increased; the thromboxane A2 (thromboxane A2 TXA2)/I2 (prostaglandin I2, prostaglandin PGI2) can contract renal arterioles, decrease glomerular filtration rate, while PGI2 and PGE2 (prostaglandin E2, PG E2) is caused by on the role of TXA2. In patients with severe hepatitis with IETM, elevated systemic levels of PGI2, reducing the renal vascular resistance, leading to renal vascular resistance TXA2, reduce the renal blood flow and glomerular filtration rate, promote the formation of HRS; third, nitric oxide, nitrogen monoxide) NONO can through vasodilatation of the systemic, resulting in effective circulating blood volume reduction and evoked HRS; endothelial endothelin (ET) et caused renal cortical blood priming HRS; and platelet activating factor (growth factor (PAF) endotoxin and platelet activating factor (PAF) can lead to a decrease in cirrhotic rats cardiac ejection fraction, reducing blood flow to the kidney, and PAF antagonist can improve hemodynamics changes [353].

Hepatic Encephalopathy

The mechanism of endotoxin induced hepatic encephalopathy is not clear. We have known that LPS can increase the permeability of blood brain barrier, promote intestinal toxic substances through the blood brain barrier (BBB), damage mitochondrial oxidative metabolism in brain cells, reduce oxygen utilization in patients with liver cirrhosis and disrupt energy metabolism of brain cells, induce brain edema.

The clinical symptoms of chronic severe hepatitis with endotoxemia included in addition to fatigue, anorexia, tiresome of the oil, nausea, vomiting, yellow skin and sclera and, performance of endotoxemia. Endotoxin can cause the release of histamine, 5-hydroxytryptamine (5-HT), prostaglandin, bradykinin, resulting in micro circulation expansion, venous blood volume reduction, decreased blood pressure, inadequate tissue perfusion, hypoxia and acidosis, and main symptoms and signs are: fever, elevated white blood cell count, bleeding tendency, heart failure, renal dysfunction, hepatic injury, nervous system symptoms and shock [354].

2.7.1.4 The Prevention and Treatment of Endotoxemia

Improve Liver Function This is the basic treatment of IETM. Liver function improved can strengthen mononuclear phagocyte system function to help the endotoxin removal. It can also decrease portal vein pressure to relieve intestinal congestion, edema, hypoxia, improve the intestinal microenvironment, reduce production and lymph reflux, and lower door shunt. These all contribute to the prevention and treatment of IETM.

Reduction in the Generation and Absorption of Intestinal Toxins

Clean the Gut

Saline is available as enema if severe liver disease, which helps reduce intestinal endotoxin generation and absorption.

Adjust the Gut Microflora Environment

Decompensated cirrhosis is often accompanied by small intestinal bacterial overgrowth and intestinal flora disturbance. Thus, the promotion of intestinal flora back to normal state help prevent and treat intestinal endotoxemia. A variety of Bifidobacterium, Lactobacillus can be selected.

Lactulose

A synthetic disaccharide, it is not digested and absorbed in the small intestine, but can be broken down into lactic acid, acetic acid and other small molecules by the bacteria into the colon. Such acidification of the intestine reduces the generation and absorption of endotoxin, and promotes the growth of intestinal bacteria, stimulates bowel movements so as to increases tool frequency and so on. In addition, the lactulose may have internal direct inactivation of toxins, prevents activation of macrophages to release cytokines.

Application of Antibiotics

Oral absorption of antibiotics can effectively suppress the generation of intestinal endotoxemia. Patients with liver cirrhosis taking oral polymyxin E or neomycin, the level of plasma LPS and NO_2 -/NO_3- horizontal declines in synchronization. Polymyxin B has an internal direct antitoxin effect [355].

Cholic Acid

It play a role by reducing intestinal absorption of toxins, inactivating toxins and inhibiting those LPS-induced media by monocyte macrophages.

The Specific Anti-toxin Preparations

The new anti-endotoxin therapy including interrupt endotoxin synthesis, binding or neutralizing its activity, preventing its interaction with the host effector cells, or interfering with toxin-mediated signal transduction pathways. Therapeutic formulations include endotoxin analogs, antibodies, subunit vaccines, polymyxin combination column, recombinant human protein, small molecule inhibitors of endotoxin synthesis and intracellular signal transduction.

Inhibition of Lipid A Biosynthesis

Bacteriophage producing a piece of short nucleotide sequence which plays the role of an antisense RNA, blocking the synthesis of bacterial LPS synthase. Current clinical studies carried out an experiments in cloning of human anti-endotoxin lipid A light and heavy chain variable region. It laid the foundation for the next screening and expression that recombination between DNA of antibodies and phage's succeeded. The Clone45 is one kind of anti-polymyxin B (polymyxin B) monoclonal antibody of the IgM class. It can play the role of anti-endotoxin shock by imitating

the surface antigen structure of lipid A so as to substitute receptor antagonist of lipid A and LPS blocking the causative link that the endotoxin induces inflammatory mediators [356].

Anti-endotoxin Antibodies and Vaccines

Isolating antibodies having a high affinity of various G-bacteria to prepare a chimeric monoclonal IgG1 antibody SDZ219-800 which have a therapeutic effect on the human endotoxemia. Anti-endotoxin core glycolipid monoclonal antibody (anti-monoclonal antibody R595) can prevent and treat the metabolic disorders in peritoneal infection with MODS; it plays a significant role in conditioning in high catabolism, and can significantly improve metabolic disorders under the condition of abdominal infection associated with MODS.

Bactericidal/Permeability-Increasing Protein (BPI)

BPI is a human endogenous protein, found primarily in neutrophils primary particles. Its molecular amino-terminal and carboxy-terminal appear V-shaped structure planar symmetry. Many amino acids to form a hydrophobic capsule hold LPS's lipid A. It was reported that BPI has an obvious protective effect on intra-abdominal infection induced sepsis, which might be related to its antagonism against endotoxin [357].

Reconstructing HDL (High Density Lipoprotein, HDL)

HDL can be used as an endogenous LPS scavenging system, binding of bacterial endotoxin with high affinity to form a stable HDL-LPS. LPS-HDL complexation may contribute to a reduction in endotoxic activities in vivo by preventing LPS (lipid A) from generating important transmembrane signals after binding to cells [358].

LPS Antagonists

E5531 is the first generation lipid A analogue, which is derived from the lipid A structure from the endotoxin of Rhodobacter capsulatus. It can block LPS in cell culture without any endotoxin-like activity. E5531 can protect mice from lethal doses of LPS, and viable E. coli infections in combination with antibiotics. In human healthy volunteers who are exposed to intravenous LPS. E5531 also blocks the endotoxin response [359].

An anti-CD14 Antibody

CD14 has a very important role in monocyte-macrophage cell signaling. Since epitopes of LPS on the cell membrane at the binding site is the same material with soluble CD14, so we can develop a monoclonal antibody interfering with LPS binding to CD14 and blocking to pass activation signals from immune effector cell [360].

LPS Signal Transduction Inhibitors

Tyrosine kinase and mitogen-activated protein kinase are involved in LPS cellular signal transduction.

Anti-endotoxin Single-Chain Antibody
Build anti-endotoxin (LPS) single-chain antibody gene and attempt to make it express in E, coli. The ScFv gene was successfully constructed and GST ScFv fusion protein highly expressed in E. coli was obtained.

Other Treatment

Glucocorticosteroids

Glucocorticoids, including the synthetic glucocorticoid dexamethasone, are recognized for their anti-inflammatory properties and have the ability to inhibit the production of proinflammatory cytokines such as TNF-α**.** In Intestinal ischemia-reperfusion methylprednisolone pretreatment can prevent endotoxemia. Combined LPS and dexamethasone treatment at 120 h significantly changed TNF**-α** [361]**.** This points that glucocorticosteroids added before or during stimulation of macrophages can prevent TNF release, after which the administration would be invalid. In fact, it is very difficult to use corticosteroids before TNF release.

TNF Blocker Therapy

Current clinical anti -TNF antibodies and TNF antagonist use exists, and before many scholars have obtained a more satisfactory results of blocking or neutralizing excessive TNF with anti-TNF. Although the clinical symptoms improved, yet the survival rate is not higher than expected. The effect of anti--TNF clinical application needs further evaluation.

MODS

Concepts Related to MODF

The first proposed concept is sequential organ failure, based on which multiple organ failure (multiple system organ failure, MSOF or MOF) is put forward, and in 1980 diagnostic criteria is developed, but it reflected the end-stage, denied reversibility, ignore the dynamic development from organ dysfunction to failure. Therefore, the US ACCP/SCCM proposed to replace the concept with MODS. MODS emphasized an early phase of organ dysfunction before overt failure occurred. It is defined as “the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.” [362] Organ dysfunction may be relative, or can be absolute; with extension of time, MODS can increase or reverse. Thus, the term.

MODS was coined to indicate the wide range of severity and the dynamic nature of this disorder, which contributes to early diagnosis and treatment of patients and is more in line with clinical practice.

There are two relatively distinct (although not mutually exclusive) pathways by which MODS can develop: In primary MODS, there is a direct insult to the organ that becomes dysfunctional. Examples of such direct insults include gastric aspiration in the lungs or rhabdomyolysis in the kidney. This direct insult causes: an inflammatory response that is localized, at least in the beginning, to the affected

organ. Secondary MODS is a consequence of trauma or infection in one part of the system that results in the systemic inflammatory response and dysfunction of organs elsewhere [363]. Secondary MODS means not directly caused by damage, but to experience the "second strike", the first blow can make the immune system in a pre-activated state under which inflammation lost control and significant SIRS appears, then the following second strike can quickly cause multiple distant organ dysfunction and easily form sepsis with the basis of SIRS/CARS. This type of MODS often develops as the following model: the original cause → Stress → immune WPW → SIRS/CARS → infection → Sepsis → MODS → MOF.

2.7.1.5 Severe Hepatitis and Multiple Organ Dysfunction

The process of severe hepatitis, can cause intestinal damage and massive release of endotoxins into the blood, leading to intestinal endotoxemia (IETM). Endotoxin is a powerful trigger for complement activation, and then these complement can activate "cascade effect" (Cascade) to release oxygen free radicals, prostaglandins, endorphins, PAF, cytokines and other inflammatory mediators to cause cytotoxicity, the error of microcirculation and tissue metabolism, eventually leading to the occurrence of MODS. In this case, we emphasize MODS originated in continuous, uncontrolled inflammation and factors causing systemic inflammatory response can be induced MODS, including bacteria, fungi, parasites, viruses, toxins and other infectious agents.

Myocardial Damage

It is a debate of long-century problems that whether endotoxin has effects on hearts. The late 1970s a large number of experiments prove endotoxins has a role of heart. It enables reduction of coronary blood flow, decrease of total coronary vascular resistance, and have meanings in heart failure. Studies have shown that endotoxin can damage myocardial mitochondria, muscle paddle net, muscle membrane, contractile proteins etc., leading to cell membrane system damage, energy metabolism. Past research in cardiac dysfunction under endotoxin shock did not attract enough attention, for they think that the heart is the final failure among all organs so that clinical treatment value is little. Now people's awareness has completely changed that heart dysfunction can occur early in sepsis or septic shock. Therefore, early identification and prevention of the occurrence and development of cardiac dysfunction may have some clinical value for treatment of septic shock and other serious complications [364].

Lung Injury

Clinical Gram-negative bacteria sepsis often complicated by adult respiratory distress syndrome, or server development of MODS. In this pathological process, the high biological activity of endotoxin plays an important role. Endotoxins often involving the lungs firstly, and the pathogenesis of non-injury may be associated with the direct damage on endothelial cell by endotoxin through complement pathway and induction of cytokines [365].

Liver Damage

The liver is the major site of endotoxin on clearing and detoxifying and the place where clinical Gram-negative bacteria sepsis often can be complicated, and it is also the primary organ suffering attacks by toxins. It is generally believed that toxins in the liver circulating mainly from the gut and liver dysfunction is closely related to the formation of endotoxemia. Histological examination revealed the inner toxins damage the liver cells, showing sinusoidal congestion, dieldrin expansion chamber, Kupffer cell swelling, endoplasmic reticulum、mitochondria swelling, crest destroy and lysosomal activation etc. [366].

The liver is the body's largest metabolic organ, and many cases of acute liver failure often involve other organ complications, which has become an important factor in determining the prognosis. Endotoxins may cause the reduction of liver nutritional blood flow, mitochondrial oxygen metabolism, interfering with sugar metabolic pathways in liver leading to metabolic disorders. Various damaging factors (such as gastrointestinal disorders, ischemia, immunocompromised, dysbiosis) promote absorb of intestinal bacteria and toxins and displacement via the portal vein, the lymphatic system into the systemic circulation. On the one hand these infectious agents can directly damage liver cells, or mediate hepatic injury whether by Kupffer cell; on the other hand it can induce systemic inflammatory response by the monocyte-macrophage cells to release the media, both leading to organ perfusion disorder, affecting protein synthesis and energy metabolism, eventually resulting in severe sepsis, MODS and even death.

Renal Dysfunction

The effect of endotoxin on kidneys is not clear yet. In the early stage, it can affect the kidneys, decreased its blood flow renal via vasoconstriction. When endotoxemia is complicated by renal failure, the mechanism of glomerular filtration rate decreasing is unclear. The pathophysiology of AKI in sepsis is complex and multi-factorial and includes intrarenal hemodynamic changes, endothelial dysfunction, infiltration of inflammatory cells in the renal parenchyma, intraglomerular thrombosis, and obstruction of tubules with necrotic cells and debris [367].

Disseminated Intravascular Coagulation

The relationship between endotoxin and DIC is quite complicated. DIC is considered to be an important incentive of MODS, especially patients with severe sepsis with DIC having a highly possibility of developing MODS, what's more, the prognosis is very poor, and the mechanism is multifaceted. Endotoxin can start the endogenous coagulation system directly or via activating factor XII (Hageman factor) by damaged endothelial cells, also can act on the monocyte-macrophage cells, stimulate the release of tissue factor to trigger the extrinsic coagulation pathway [368].

Gastrointestinal Injury

In clinical practice it has been noted that serious infections is very possible to be complicated by gastrointestinal failure. The intestine is the biggest reservoir of

bacteria in the body and leakage of bacteria or microbial products, notably LPS, from the lumen of the gut into the systemic compartment, leads to initiation or amplification of a deleterious inflammatory response and MODS [369]. After endotoxins challenging the gastrointestinal mucosa, it initially shows mucosal telangiectasia, interstitial edema and hemorrhage. Microcirculation leading to damage of lysosomal and release of proteases in the cell, cell degeneration and necrosis. In addition, mucosal cellular energy metabolism decrease, H + reverse diffusion, prostaglandins and bradykinin further aggravate mucosal damage. At the same time destroy of the gastric mucosal barrier also make a lot of bacteria and endotoxins pass through the gastrointestinal mucosa, migrate to the blood circulation, the lymphatic system and the abdominal cavity etc., leading to systemic multi-system organ damage.

2.7.1.6 MODS Clinical Manifestations

Clinical Characteristics of MODS

1. Organ failure usually do not result directly from the primary injury. There is a certain time interval from the primary injury to organ failure.
2. Not all of infection have bacteriological evidence, and more than 30% of patients and autopsy found no infected lesions. Thus, to identify and treat the infection may not be able to improve the patient's survival.
3. MODS may have perfectly healthy organ involved, and it is ferocious and rapidly progresses. Once happened, it is difficult to depress in the event almost, so often with a high mortality rate.
4. In pathology, MODS lacks features, the affected organ only showing acute inflammation, such as inflammatory cell infiltration and so on, and these changes are very inconsistent with severe clinical manifestations, and once restored, patients do not have any clinical sequelae.
5. MODS is closely with shock and infection. Shock, infection, injury (including trauma and surgery, etc.) are the three main causes of MODS.
6. Generally the later period of shock will typesetting IDC and MODS, and the order of occurrence of MODS usually is the lungs, liver, kidney, gastrointestinal tract, finally the heart.

The Characteristic Clinical Manifestations

1. **Instability of circulation** due to a variety of inflammatory mediators have effects on the cardiovascular system, the circulation is most likely involved. Almost all cases, at least in the course of the early and middle will be in high-power type of cycle of "high ranked low resistance". Cardiac output up to 10 L/min or more and low peripheral resistance cause shock and need vasopressors to maintain blood pressure.
2. **High metabolic** systemic infection and MODS are usually accompanied by severe malnutrition. Its metabolic mode has three salient features: (1) persistent high metabolism, metabolic rate up to 1.5 times more than normal; (2) abnormalities of energy pathway. In starvation, the body obtain energy mainly through

the decomposition of. However, with systemic infection, the body will get energy by breaking down proteins while the use of sugar is limited and fat utilization may increase early, fall later; (3) poor response to exogenous nutrient, supplement of exogenous nutrition can not effectively prevent itself consumption, which suggests that a high metabolism itself has a "mandatory" also known as "autophagy Metabolism." High metabolic may have serious consequences. First, protein malnutrition result from it will cause serious damage to the structure and function of the enzyme system of organs; secondly, imbalance of branched-chain amino acids and aromatic amino acid which makes the latter formate into a pseudo-neurotransmitter, then further lead to dysfunction of nerve.

3. **Hypoxia in tissue cells** at present many scholars believe that the high metabolic and circulatory disorders often cause oxygen supply and oxygen demand does not match, so that the tissues of bodies are in a hypoxic state, mainly clinically manifesting "oxygen supply dependency" and "lactic acidosis. ".

2.7.1.7 Diagnosis of MODS

Currently MODS still lacks an unified diagnostic criteria, and any one of the diagnostic criteria of MODS is difficult to reflect the entire contents of organ dysfunction, so in clinical practice we can select one according to our own specific situation.

1. The main contents from National Critical Care Medicine Conference standard In 1995 are: (1) respiratory failure: R > 28/min; PaO_2 < 6.7 kPa; PCO_2 > 5.89 kPa; $PaO_2/FiO_2 \leq 26.7$ (200 mmHg); P (Aa) DO_2 ($FiO_2$1.0) > 26.7 kPa (200 mmHg); X-ray of chest shows alveolar consolidation ≥1/2 lung (which have more than three or three); (2) renal failure: except prerenal factors, little or no urine, serum creatinine, increased blood urea and nitrogen levels, exceeding more than twice the normal value; (3) heart failure: systolic blood pressure < 80 mmHg (10.7 kPa), sustained more than 1 h; CI <2.6 L/(min · m^2); ventricular tachycardia; ventricular fibrillation; degree atrioventricular block; resuscitation after cardiac arrest (with which three or more); (4) liver failure: total bilirubin>34 μmol/L; liver enzymes increased more than 2 times compared with the normal; prothrombin time > 20s; with or without hepatic encephalopathy; (5) DIC: platelets 100 × 10^9/L; prothrombin time and partial thromboplastin time prolong 1.5 times, and fibrin degradation products increase; systemic hemorrhage; (6) Brain failure: Glasgow score below 8 means coma, and less than 3 points means brain death.
2. The sooner the primary diseases or the primary risk factors are eliminated or controlled, the greater the possibility of organ recovery is.

2.7.1.8 Treatment Principles

1. To effectively rescue and debride as soon as possible, prevent infection, prevent ischemia-reperfusion injury, use a variety of supportive care;
2. To reduce stress response, mitigate and shorten high metabolism and the magnitude and duration of glucocorticoid receptor;

3. To pay attention to the patient's breathing and circulation, as soon as possible to correct hypovolemia and hypoxia;
4. To prevent infection is an important measure of preventing MODS;
5. If possible, improve the nutritional status of patients.
6. Early treatment of any starting organ failure.

MODS is a problem in the medical field with an acute onset, rapid progression, and high mortality rate. So far for MODS, there is no specific treatment, but through clinical monitoring, early detection of possible organ dysfunction, early intervention, and taking effective measures can slow down or block the course, improve the success rate.

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