Mechanisms of Acute Liver Failure

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Introduction

Acute liver failure (ALF) is characterized by the sudden onset of liver failure in a patient with no evidence of chronic liver disease. This definition is important as it differentiates patients with acute liver failure from patients who suffer from liver failure due to end-stage chronic liver disease (decompensated cirrhosis and acute-on-chronic liver failure, ACLF).

ALF is a rare condition and affects about 2000 persons per year in the USA. It is defined as severe hepatopathy with elevated transaminases twofold the upper limit of normal, liver dysfunction (icterus and coagulopathy with an international normalized ratio (INR) >1.5), and hepatic encephalopathy.

Chronic liver disease and the secondary causes of liver dysfunction, such as sepsis and cardiac shock, have to be ruled out. Nevertheless, acute decompensation of Wilson’s disease, reactivation of chronic hepatitis B, and autoimmune

Key Points

• Acute liver failure is characterized by the sudden onset of liver failure in a patient with no evidence of chronic liver disease.
• Four different mechanisms are mainly responsible for ALF: (1) infectious (mostly viral), (2) drugs/toxins/chemicals, (3) cardiovascular, and (4) metabolic.
• Suicidal acetaminophen ingestion is the most frequent cause of drug-induced liver failure.
• Three factors determine the prognosis of liver failure: (1) the metabolic consequences resulting from liver failure, (2) the release of mediators and toxic metabolites, and (3) the capacity of the remaining hepatocytes to restore liver mass.
• Cerebral edema, infections, and multiorgan failure are important clinical complications that limit patient survival.
• Ammonia levels can be used for risk stratification in patients with acute liver failure and subsequent hepatic encephalopathy.
• Intravenous administration of N-acetylcysteine improves transplant-free survival of patients with early-stage non-acetaminophen-related acute liver failure.
• Treatment with high-volume plasma exchange improves the outcome of patients with ALF by increasing liver transplant-free survival, potentially by attenuating innate immune activation and ameliorating multiorgan dysfunction.
• Cytokines are involved in the pathogenesis of acute liver failure and in controlling the balance between survival and hepatocyte proliferation.
• The mode of liver cell death that is predominantly induced in ALF (apoptosis or necrosis) is determined by the underlying etiology, the duration of the disease, and the extent of liver injury.
• Future characterization of the molecular cell death mechanisms might establish potential diagnostic and therapeutic targets in ALF.
• Intestinal dysbiosis has been recently identified as a driver of ALF severity. The understanding of gut-liver interaction during ALF might facilitate innovative therapeutic interventions.
hepatis – in fact, chronic liver diseases – are considered as cases of acute liver failure.

Other common clinical features of ALF are cardiovascular instability, susceptibility to infection, acute kidney injury, and cerebral edema. Owing to the affection of all organ systems, ALF is associated with an overall mortality of approximately 30%. ALF accounts for 6–8% of liver transplantations in the USA and Europe [1]. The data of the US ALF study group are presented in Fig. 29.1; spontaneous survival occurs in approximately 45%, liver transplantation in 25%, and death without transplantation in 30% of adults with ALF [1].

The time between the first symptoms and the manifestation of hepatic encephalopathy has been shown to be crucial for the prognosis of these patients. Therefore, several groups have included in their definition the time frame between the onset of symptoms and the start of encephalopathy.

The definition of the US ALF Study Group (ALFSG) uses the term acute liver failure as an umbrella and differentiates the three subgroups: hyperacute, acute, and subacute (Fig. 29.2). The time between the first symptoms and encephalopathy in hyperacute ALF is 7 days; in acute ALF, it is 8–28 days; and in subacute ALF, it is 5–26 weeks [2].

Hepatocyte injury can be caused by direct toxic necrosis, often related to hyperacute ALF, or by apoptosis and immune injury, which is a common feature of acute and subacute ALF.

Typically, in hyperacute ALF, very high aminotransferase concentrations and low bilirubin concentrations can be observed, whereas in acute and subacute ALF, lower aminotransferase levels and higher bilirubin levels are common. In general, patients with hyperacute liver injury have better short-term survival in comparison with patients with slowly progressing liver injury. Nevertheless, the cause of hepatic injury has superior prognostic potential as compared with time frame to evolve ALF.

**Mechanisms of Disease**

There are different causes of ALF. In principle, four different classes can be differentiated: (1) infectious (mostly viral), (2) drugs/toxins/chemicals, (3) cardiovascular, and (4) metabolic [3] (Table 29.1). In developed countries, acetaminophen toxicity, ischemia, drug-induced liver injury, hepatitis B, and autoimmunity account for nearly 80% of the cases [4].

There are obvious differences in the mechanisms that initially trigger liver failure. However, at the time of clinical presentation, in most cases, a common final stage has been reached in ALF patients. At this stage, three main factors seem to be important in determining the prognosis: (1) the

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**Table 29.1** Causes of acute liver failure

| Infectious causes | Rare causes of infectious etiology |
|-------------------|---------------------------------|
| Hepatitis A-E     | Herpes simplex virus types 1 and 2, Human herpes virus type 6, Varicella virus, Cytomegalovirus, Epstein-Barr virus, Parvovirus B19, Toxoplasma, Paramyxovirus, Parainfluenza virus |

| Drugs and toxins | Cardiovascular syndromes |
|------------------|-------------------------|
| Acetaminophen, halothane, isoniazid, valproate, tetracycline, nonsteroidal anti-inflammatory drugs (NSAIDs), pirprofen, ketomazole | Budd-Chiari syndrome, hypotension (circulatory shock, left ventricular failure), heart failure (e.g., right ventricular failure, valvular heart diseases), hyperthermia, malignant tumors, veno-occlusive disease, portal vein thrombosis, sepsis |

| Metabolic diseases | Wilson’s disease, Reye’s syndrome, acute fatty liver of pregnancy (AFLP), HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count), galactosemia, hereditary fructose intolerance, hereditary tyrosinemia |

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**Fig. 29.1** Natural history of ALF. Liver regeneration with spontaneous survival occurs in approximately 45%, liver transplantation in 25%, and death without transplantation in 30% of adults with ALF. (Data from the United States); LTX liver transplantation [1]

**Fig. 29.2** Definition of ALF. ALF is defined as a severe liver injury, clinically characterized by coagulopathy and hepatic encephalopathy within 26 weeks of symptom onset in previously healthy subjects
metabolic consequences resulting from the loss of liver cell mass, (2) the release of mediators and toxic metabolites from the liver tissue, and (3) the capacity of the remaining vital hepatocytes to restore liver mass [5, 6].

Therefore, in terms of the mechanisms that are important during ALF, two different phases of ALF can be differentiated: the mechanisms that initially trigger liver failure and those that eventually determine the outcome.

The etiology of ALF and coma grade on admission are two prominent factors influencing prognosis. ALF caused by acetaminophen toxicity, hepatitis A, ischemia, and pregnancy are associated with at least 60% short-term transplant-free survival, whereas drug-induced liver injury, (reactivation of) hepatitis B, autoimmune hepatitis, and indeterminate causes are associated with a spontaneous recovery rate of only 30% [7]. Patients presenting with early grades of hepatic encephalopathy in ALF (independent of etiology) usually have a more favorable outcome than those with established stupor or coma [8]. Liver transplantation, intensive care medicine, and specific therapeutic options (Table 29.2) can improve prognosis [9].

### Etiology

#### Infectious Causes

Viruses in particular are an essential cause of ALF and, depending on the geographical region can comprise between 30% and 70% of all forms of ALF [3, 5, 6]. In the developing world, infections with hepatitis A, B, and E viruses are accounting for most cases of ALF. In Europe, the data from the ELTR database reveal that liver transplantation for ALF due to HAV and HBV decreased significantly in the last 5 years (from 1% to 0.5% and from 17.9% to 13.2%, respectively) [10].

### Table 29.2 Specific therapeutic options in ALF

| Cause of ALF          | Treatment                        | Dosage          |
|-----------------------|----------------------------------|-----------------|
| Acetaminophen         | N-acetyl cysteine                | 300 mg/kg       |
| Amanita poisoning     | Silibinin                        | 20–50 mg/kg/day |
| Acute hepatitis B     | Lamivudine, Entecavir, Tenofovir | 100–300 mg/day  |
|                       |                                  | 0.5–1 mg/day    |
|                       |                                  | 245 mg/day      |
| HELLP/AFLP            | Termination of pregnancy         | –               |
| Autoimmune hepatitis  | Prednisolone                     | 1–2 mg/kg/day   |
| Budd-Chiari syndrome  | TIPS/surgical shunt              | –               |
| Herpes simplex hepatitis | Aciclovir                      | 3x10 mg/kg/day  |

Modified from Ref. [9]

TIPS: transjugular intrahepatic portosystemic shunt shunt

Hepatitis A Virus

Due to effective use of vaccination, infections with the hepatitis A virus (HAV) have declined over the last decade and accounted for 3% of the ALF cases in the USA [11]. The proportion of patients with ALF is higher in older than in younger patients. This is relevant, as in Western countries over the last decades, HAV infection has occurred more frequently in older patients, and thus, the risk of ALF increases in this population [12, 13]. Recent widespread outbreaks of hepatitis A infections among homeless people in the USA resulted in an increased rate of hospitalizations and demonstrated a lack of vaccination in the general population [2]. Furthermore, patients with underlying chronic liver disease, especially chronic hepatitis C, have an increased risk of ALF in the context of HAV infection [14].

The pathogenesis of HAV-related ALF is not completely understood. Current studies indicate that a combination of a direct cytopathic effect of the virus and immune-mediated mechanisms results in liver destruction. In comparison with other hepatotropic viruses, ALF caused by hepatitis A has a favorable prognosis with spontaneous or transplant-free survival of nearly 70% [11].

Hepatitis B Virus

The risk of acute liver failure of all patients who are hospitalized due to an acute hepatitis B virus (HBV) infection is around 1% [15]. Fulminant HBV is the most predominant viral cause of ALF in Western countries [7, 16] and accounts for 7–10% of ALF in Europe and 7% in the USA [1, 10]. Due to the implementation of routine vaccination, the incidence of fulminant HBV has decreased. In fulminant acute HBV infection, antiviral therapy with lamivudine, entecavir, or tenofovir has been proven efficient and safe, with a significant reduction in HBsAg concentrations (see Table 29.2) [17, 18]. Once ALF is established, the clinical benefit of antiviral therapy is not proven. Nevertheless, antiviral therapy should be given to transplantation candidates, since viral suppression prevents HBV recurrence after following transplantation [19].

Approximately two-thirds of the cases of ALF due to hepatitis B are caused by new infections, and the remainder are caused by reactivation of (unrecognized) chronic hepatitis B infection in the setting of chemotherapy or immunosuppression. Reactivation of HBV or infection with highly replicative HBV harboring precore and core-promoter gene mutations became a more important cause of ALF [20, 21].

Virus reactivation is associated with a much higher risk of ALF than is novel acute HBV infection, and antiviral prophylaxis should be administered to HBsAg-positive patients who are about to receive chemotherapy or immunosuppressive therapy [22, 23]. Clinical differentiation of ALF due to acute or chronic hepatitis B infection is often difficult if there is no previous history of hepatitis B infection. Quantitative measurements of immunoglobulin M (IgM) anti-hepatitis B
core antibody (anti-HBc) titers and HBV viral loads might allow etiological discrimination [24].

In general, the HBV itself is not cytopathic, but the immune response directed against the virus is essential [25]. Frequently at the time of hospitalization, the viral load is already decreasing, whereas transaminases are still increasing. This may reflect the possibility that different factors contribute to the elimination of the virus. The data indicate that cytokines – namely, interferon (IFN) – are operating through a noncytopathic mechanism to eliminate the HBV genome in hepatocytes, whereas at a later stage, the T cells infiltrate the liver and destroy the hepatocytes [26]. Therefore, the activation of HBV-specific T cells is important to determine the degree of hepatic injury during ALF.

In the case of HBV/hepatitis D virus (HDV) coinfection, the risk of ALF increases [27]. The exact mechanisms that lead to more pronounced liver failure are not defined.

**Hepatitis C Virus**

The risk of ALF through hepatitis C virus (HCV) is very low [5]. In Japan, in particular, cases of HCV-related ALF have been documented [28]. As there are only a few reports in the literature, the pathogenesis of HCV-related ALF is not completely understood. However, there is evidence that elimination of HCV-specific T cells is associated with chronic HCV infection [29]. This indicates that the HCV-specific immune response is involved during acute infection and thus is most likely the determining factor during ALF.

**Hepatitis E Virus**

Acute liver failure owing to hepatitis E virus (HEV) infection is seldom observed in Western countries. However, hepatitis E has a predilection for older men in whom it causes substantial morbidity and mortality [30]. Based on a poor prognosis in combination with preexisting liver disease, patients with unexplained hepatitis should be tested for HEV [31]. Epidemic outbreaks are known in developing countries, especially in patients with ALF. In India, Pakistan, China, and Southeast Asia, HEV infection is the most predominant cause of ALF [31]. Pregnant women, especially in the third trimester, have been regarded to have a high risk for ALF (up to 20%) [32].

However, recent data indicate that pregnancy does not affect the outcome of ALF resulting from HBE infection [33]. The mechanisms operating in patients with HBE infection-induced ALF have not yet been sufficiently studied. Therefore, there is no clear hypothesis in the literature, and it is only speculative to draw parallels with HAV.

**Rare Cases of Viral Hepatitis**

In rare cases, different systemic virus infections can present as ALF owing to a predominant manifestation in the liver. These are the herpes simplex virus types 1 and 2 (see Table 29.2), human herpes virus type 6, cytomegalovirus, varicella-zoster virus, Epstein–Barr virus, and parvovirus B19. A few cases of ALF related to an infection with the togavirus, paramyxovirus, and parainfluenza virus have also been described.

**Drugs, Toxins, Chemicals**

Drug toxicity is the predominant cause of ALF in Western countries. Several drugs, chemicals, and toxins can cause ALF (see Table 29.1) by either direct toxicity or idiosyncratic drug reaction. The most frequent examples are discussed in this review.

**Acetaminophen**

Acetaminophen (Paracetamol, Tylenol) is the most common cause of ALF. In adults, only higher doses (in general, more than 10–12 g) are dangerous, and in most cases, acetaminophen was taken in a suicide attempt. Patients who consume alcohol chronically and those with non-alcoholic fatty liver disease (NAFLD) may be more susceptible for acetaminophen toxicity, as cytochrome P450 has been induced in their liver [34].

Measurement of serum acetaminophen-protein adducts (toxic byproducts of cell injury: acetaminophen bound to cell proteins) can reliably identify acetaminophen toxicity in cases of ALF, in which no clinical or historic data are given that would reveal the cause up to 3 days following ingestion [35, 36]. At present, these analyses are only available in specialized laboratories. Acetaminophen toxicity causes 46% of the cases of ALF in the USA and 65% in the UK [37, 38].

The pathogenesis of acetaminophen injury is related to the formation of toxic metabolites through the cytochrome P450 enzymes, especially cytochrome P450 2E1 [39, 40]. These toxic metabolites are normally conjugated and inactivated through glutathione. However, when glutathione stores are depleted, these toxic metabolites accumulate, resulting in hepatocyte injury (Fig. 29.3).

The pattern of hepatic injury in acetaminophen toxicity is similar to ischemia, with a rapid-onset necrosis beginning 8–12 h following ingestion. The typical clinical features are very high levels of aminotransferase and elevated INR but normal or slightly increased bilirubin levels. Peak levels are expected at approximately 72 h. Necrosis has been shown to be the more prominent form of cell death in acetaminophen toxicity [41]; however, in vitro data and animal data suggest that apoptosis also contributes to acetaminophen-induced ALF [42–44].

In fact, it has been demonstrated that the course of disease in acetaminophen-induced liver failure is on the one hand influenced by the acetaminophen dose and the initial hepatocyte damage and on the other hand by the inflammatory...
response following acetaminophen-induced liver failure. Necrotic hepatocytes release danger-associated molecular patterns (DAMPs) which are recognized by hepatic macrophages, Kupffer cells, and neutrophils and consecutively result in the activation of these cells. The detection of DAMPs and pathogen-associated patterns (PAMPs) is exerted by the inflammasome, a highly regulated signaling system in myeloid cells, which conclusively leads to the activation of monocytes and neutrophils by the release of pro-inflammatory cytokines, interleukin (IL)-1ß, and IL-18 through a proteolytic cleavage pathway mediated by the activation of caspase-1 [45]. Also, activated macrophages release pro-inflammatory cytokines (e.g., TNF-α, IL-1ß, and IL-18) as well as chemokines (e.g., CCL2), thereby enhancing hepatic inflammation. Additionally, monocytes which are mainly recruited by their receptor CCR2 further aggravate inflammation. Those liver-infiltrating monocytes can mature into monocyte-derived macrophages (MoMF), which are involved in the resolution of inflammation. Natural killer T cells are additional parts of the inflammatory response to acetaminophen toxicity and may maintain hepatic inflammation [46].

N-acetylcysteine (NAC), the standard antidote for acetaminophen overdose, exerts its therapeutic effects by restoring the depleted hepatic glutathione stores and is usually given at a cumulative dose of 300 mg/kg BW over 21 h (see Table 29.2) [47].

A recent multicenter study from Australia (NACSTOP) has shown that shortening of the NAC regimen for acetaminophen poisoning is possible in selected low-risk patients. Low risk was defined as normal ALT levels at baseline and after 12 h, and acetaminophen level <20 mg/l at 12 h. In this cohort, reduction of the NAC regimen to 12 h with a total NAC dose of 250 mg/kg BW was safe [48].

Moreover, intravenous NAC improves transplant-free survival in patients with early-stage non-acetaminophen-related ALF. However, patients with advanced coma grades do not benefit from NAC and typically require emergency liver transplantation [49].

**Mushroom (Amanita) Poisoning**

Mushroom poisoning, mainly through the species *Amanita phalloides* (tuber toadstool) frequently leads to ALF, especially in fall. The clinical spectrum of *Amanita* poisoning varies from acute gastroenteritis to the development of ALF.

After ingestion of the tuberous toadstools, there is initially a symptomless latency phase for 5–24 h, until vomiting, massive diarrhea, abdominal colic, and exsiccosis are in the foreground in the gastrointestinal phase over a period of 12–24 h. In this phase, tuber-toed mushroom intoxication is often misinterpreted as “food poisoning” or gastroenteritis. This is followed by the hepatorenal phase after 2–3 days, which is characterized by an increase in transaminases and evolving coagulopathy, icterus, and liver and kidney failure. A deleterious course can be prevented by liver transplantation or a spontaneous restitution of the liver function taking place within 2–3 weeks [50].

The toxic agent of tuber toadstool poisoning is the amanita toxin (amanitin). It mainly blocks RNA polymerase II and thereby inhibits transcription and protein biosynthesis and leads to cell death. The result is a multiorgan failure, especially the liver, kidney, and brain.

The foreground of the therapy is the primary elimination of toxins with repeated administration of activated carbon,
since the amanita toxin undergoes an enterohepatic cycle. As an antidote, silibinin can prevent the uptake of amanitin into liver cells and improve biliary excretion [51]. Although there no controlled trials proving its efficiency, silibinin is used in Europe owing to its cytoprotective effects against the amanita toxin and has been reported to be more effective than penicillin G in the amanita poisoning (silibinin is not available as a licensed drug in the USA) (see Table 29.2) [51, 52].

There are more than 1300 case reports on the clinical efficacy of silibinin as an antidote in tuber toadstool poisoning. Based on these case reports, an initial dose of silibinin of 5 mg/kg BW intravenously (I.V.) for 1 h, followed by a continuous application of 20 mg/kg BW/d I.V. until liver function has recovered, seems to be indicated. Concomitant therapy with NAC 300 mg/kg BW over 20 h I.V. potentially exerts additive positive effects [51].

Despite the advances in intensive care therapy, the mortality rate in patients who develop ALF following amanita ingestion is high [52].

A recent animal study has investigated the effects of combined antidote therapy with polymyxin B and methylprednisolone in amanita intoxication [53]. The rationale for the use of these substances is, on the one hand, that polymyxin B can reverse the inhibition of RNA polymerase II caused by amanitin. On the other hand, methylprednisolone is an inhibitor of the Na+-taurocholate cotransporter polypeptide (NTCP), which also mediates the toxic effects of amanitin and has anti-inflammatory effects.

The experimental animals received 0.33 mg/kg of amanitin intraperitoneally (I.P.) 4 h after the application of 2.5 mg/kg of polymyxin B and 10 mg/kg of methylprednisolone as antidotes I.P. Under this combination therapy, all animals survived the amanitin intoxication; without antidote, only 40% survived. The antidote combination of polymyxin B and methylprednisolone may be a new therapeutic option in tuber-toed mushroom poisoning.

It should be noted, however, that currently, there are only animal experimental data, and no dose information or suggestions for use in humans have been proposed. However, due to the expected therapeutic safety of polymyxin B and methylprednisolone, this approach seems promising.

**Halothane**

Halothane is the prototype of an idiosyncratic drug reaction that (less frequently) can also be found after anesthesia with other members of the same family. In general, halothane-related ALF is only found after the second exposure to the drug. Halothane hepatitis is a paradigm for immune-mediated adverse drug reactions, The mechanism appears to be related to the development of sensitization to both autoantigens, including CYP2D6, and halothane-altered liver cell determinants [54]. For the pathogenesis of the disease, specific antibodies are involved in hepatic injury. These antibodies can only be determined in specialized laboratories.

**Cardiovascular Disorders**

Cardiovascular diseases can lead to ALF, either by ischemia or by impaired blood flow leaving the liver. Examples of ischemic events are severe hypotension or heart failure. Aminotransferase concentrations ≥3000 U/l and bilirubin levels <5 mg/dl are strong indicators of either hepatic ischemia or acetaminophen toxicity. Ischemic hepatic injury rarely requires liver transplantation [55]. Hepatic injury due to severe heart failure can be promptly diagnosed via echocardiography by assessing the left-ventricular ejection fraction. Stasis of blood flow in the liver may occur owing to malignant tumors, veno-occlusive disease, or Budd-Chiari syndrome.

**Budd-Chiari Syndrome**

Classically, Budd-Chiari syndrome is characterized by a symptomatic occlusion of the hepatic veins and is more frequently observed in females [56]. Depending on the disease progression, Budd-Chiari syndrome may result in ALF when a sudden occlusion of at least two main liver veins occurs. Typically, acute Budd-Chiari syndrome presents with ascites, abdominal pain, jaundice, and hepatomegaly [57]. Budd-Chiari syndrome is frequently associated with primary myeloproliferative disorders, a factor V Leiden mutation, antcardiolipin antibodies, and protein C and S deficiency, which increase the risk of thrombotic complications [58]. In general, the course of disease in Budd-Chiari syndrome leads to liver transplantation. Transjugular intrahepatic portosystemic stent shunt (TIPSS) or percutaneous transjugular direct portocaval shunt, in patients with inaccessible hepatic veins, seems to be therapeutic options to decrease the portal pressure gradient, improve synthetic functions, reduce transaminase levels, and control ascites (see Table 29.2) [59, 60].

**Metabolic Disorders**

Different metabolic disorders may present as ALF, for example, Reye's syndrome, which is more common in children; its frequency has declined over the last decades. Also, during pregnancy, acute fatty liver of pregnancy (AFLP) or HELLP syndrome (hemolysis, elevated liver enzymes, and a low platelet count) may develop. Patients with HELLP syndrome typically present with LDH, ALT, and increased bilirubin level. Immediate termination of pregnancy and delivery usually reverse hepatopathy, but patients are at increased risk for complications in future pregnancies (see Table 29.2) [61].
**Wilson’s Disease**

Wilson’s disease is an autosomal recessive genetic disorder of copper metabolism and a rare cause of ALF. Wilson’s disease protein (WND, ATP7B protein) is a copper-transporting P-type ATPase and is encoded by the ATP7B gene. The ATP7B protein is located in the trans-Golgi network of the liver and brain.

ATP7B protein regulates the copper concentration level in the body by excreting excess copper into the bile and blood. Genetic disorder of the ATP7B gene (by single base pair mutations, deletions, frameshifts, and splice errors in ATP7B gene located at chromosome 13, 13q14.3) may cause Wilson’s disease, which is characterized by copper accumulation in the tissues. Hepatic disease occurs when the accumulation of copper in the liver causes mitochondrial damage and hepatocellular necrosis. The reduced excretion of copper into the bile results in increased urinary copper concentrations, leading to renal dysfunction.

The clinical appearance of Wilson’s disease comprises hepatic, renal, ophthalmic, cardiac, neurologic, and psychiatric disorders. In general, patients with ALF due to Wilson’s disease present with only moderately elevated aminotransferases and reduced levels of alkaline phosphatase but high bilirubin. Hemolytic anemia induced by copper ions leaking from necrotic hepatocytes into the circulation, causing lysis of erythrocytes, and acute kidney injury are further typical clinical features of Wilson’s disease which allow appropriate diagnosis [62]. The patients already frequently have liver cirrhosis and are therefore not in accordance with the “real” definition of ALF. However, many of the patients were healthy before the onset of the disease and therefore are categorized as patients with ALF [63].

The pathogenesis of hepatocyte injury in the context of Wilson’s disease is not completely understood. Both necrosis and apoptosis may be encountered. There is evidence that elevated copper levels are directly toxic for the cell and involve CD95-mediated apoptosis [64]. The current hypothesis postulates that excess copper generates free radicals that deplete the cellular stores of glutathione and oxidize lipids, enzymes, and cytoskeletal proteins [65].

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**Mechanisms of Organ Failure**

As a consequence of ALF, multiorgan failure (MOV) develops rapidly. Different factors contribute to MOV (Fig. 29.4).

Frequent problems that occur during this process are cerebral edema and encephalopathy, an impairment of the immune response with an increased rate of infections, coagulation disorders, and cardiovascular and kidney failure; pulmonary and metabolic complications also develop. Figure 29.5 presents an overview of the common clinically relevant complications of ALF.

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**Fig. 29.4** Mechanisms that contribute to multiorgan failure during acute liver failure

![Mechanisms of Liver Failure Diagram](image-url)
Encephalopathy and Cerebral Edema

Hepatic encephalopathy (HE) is essential for the diagnosis of ALF and is subdivided into four different grades: I–IV (Table 29.3).

In 75–80% of the patients in stage IV, cerebral edema develops independent of the cause of ALF. The treatment measures in hepatic encephalopathy comprise quiet environment, upper body elevation (30°), and, if necessary, intubation, analgesic sedation, and mechanical ventilation (at HE >3°) [67].

The precise pathophysiological mechanisms leading to hepatic encephalopathy are not completely understood [68]. However, laboratory studies indicate that the cause is an ammonia-induced deficit in neurotransmitter synthesis rather than a primary deficit in cerebral energy metabolism [69]. Most likely, the astrocytes and the pre- and postsynaptic neurons contribute to the clinical picture of hepatic encephalopathy (Fig. 29.6).

Astrocytic swelling during ALF determines the degree of cerebral edema and thus the degree of cerebral dysfunction [71].

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Table 29.3  Stages of acute hepatic encephalopathy

| Stage   | Mental state                                                                 | EEG  | GCS |
|---------|-------------------------------------------------------------------------------|------|-----|
| I, prodrome | Mild confusion, slurred speech, slowness of mentation, disordered sleep rhythm, euphoria/depression | Usually normal | 15  |
| II, apathy, lethargy | Accentuation of stage I, drowsy but speaking, inappropriate behavior, incontinence | Generalized slowing | 11–15 |
| III, stupor | Sleeps most of the time but rousable, incoherent or no speech, marked confusion | Abnormal | 8–11 |
| IV, coma | Patient may (stage IVa) or may not (stage IVa) respond to painful stimuli | Abnormal | <8  |

Modified from Ref. [66]

EEG electroencephalogram, GCS Glasgow Coma Scale
In the literature, several factors are discussed that contribute to hepatic encephalopathy, but ammonia (with a consequent dysregulation of the glutamate neurotransmitter system) seems especially relevant for the development of hepatic encephalopathy and cerebral edema. Ammonia is primarily metabolized from glutamine in the small bowel and is converted to urea in healthy liver, but in ALF, concentrations increase, and ammonia is alternatively metabolized back to glutamine.

Arterial ammonia levels at presentation have been demonstrated to be predictive of outcome in patients with ALF. Patients with encephalopathy grade III and IV exhibited significantly higher serum ammonia levels than those with lower-grade encephalopathy. Possibly, patients with advanced cerebral dysfunction can be determined by a serum ammonia cutoff value of 124 μmol/l or more. Ammonia levels can be used for risk stratification [72]. Ammonia exerts effects on cerebral function by direct and indirect mechanisms (Table 29.4).

There is clear evidence that arterial ammonia concentrations directly correlate with cerebral edema and thus herniation [73]. Experimental evidence also demonstrates that physiological ammonia concentrations alone result in astrocyte swelling. Additionally, higher glutamine concentrations are a consequence during this process, and they accelerate cerebral edema [70, 74].

Higher ammonia concentrations have a direct effect on the glutamate neurotransmitter system. Glutamate is the major excitatory neurotransmitter in the mammalian brain (see Fig. 29.6). After the release at the presynaptic neuron, glutamate binds to glutamate receptors on the postsynaptic neuron (NMDA) or on both the postsynaptic neuron and astrocytes (AMPA/KA). Additionally, glutamate transporter on astrocytes (GLT-1 and GLAST) and neurons (EAAC1) limit the expression of glutamate in the neuronal cleft. After the uptake of glutamate in astrocytes via GLT-1, it is transformed into glutamine. Ammonia downregulates GLT-1 expression on astrocytes, and this results, in higher and prolonged extracellular glutamate concentrations in patients with ALF. Additionally, there is evidence that the glutamate receptors are differentially expressed during ALF, and thus, dysregulation of the glutamate system is one of the important determinants of hepatic encephalopathy during ALF [70, 74].

Other neurotransmitters that participate in hepatic encephalopathy are GABA, serotonin, and the opioid system. Systemic circulation of pro-inflammatory mediators during ALF might also contribute to hepatic encephalopathy, as they might modulate cerebral permeability to neurotoxins, initiate inflammatory responses, and impair cerebral blood flow [75]. Hyponatremia should be avoided and corrected, as it leads to water entry into astrocytes and further promotes astrocyte swelling. Targeting serum sodium levels at 145–155 mmol/l results in lower intracranial pressure, as compared with normal sodium values of 137–142 mmol/l [76].

A few uncontrolled studies [77–79] show a protective effect of mild hypothermia in ALF and cerebral edema. Hypothermia (32–35 °C) can be safely and easily applied. The risk of complications (arrhythmias, myocardial ischemia, infections, and coagulopathy) increases with the degree and duration of hypothermia, mainly with body temperatures below 32 °C. Hypothermia reduces intracranial pressure and reestablishes disturbed autoregulation of cerebral blood flow. Some studies suggest that hypothermia can reduce the extent of liver injury in ALF [80]; in contrast, hypothermia might also lead to impaired liver regeneration.

In a prospective multicenter study, 46 patients with ALF and high-grade hepatic encephalopathy were evaluated for a protective effect of hypothermia. There was no difference in the intracranial pressure or survival between patients who were cooled to 33–34 °C compared with those cooled to 36 °C body temperature [81]. The established measures for the treatment of high-grade hepatic encephalopathy in ALF are the application of mannitol 20% (150 ml) or hypertonic saline 2.7% (200 ml) or 30% (20 ml) I.V. over 20 min and short-term hyperventilation [67, 82]. Insertion of monitors for intracranial pressure does not improve outcome but might be significant in the identification of patients who should not undergo transplantation due to prolonged intracranial hypertension or low cerebral perfusion pressure [67]. Therefore intracranial probes for measuring intracranial pressure should not be routinely used and in fact the application rate in the US and Europe is very low.

### Table 29.4 Effects of ammonia on brain function

| Electrophysiological effects of the ammonium ion |
| Effects on the inhibitory postsynaptic potential |
| Effects on glutamatergic neurotransmission |
| Effects on brain energy metabolism |
| Inhibition of α-ketoglutarate dehydrogenase |
| Effects on astrocyte function |
| Decreased expression of the glutamate transporter GLT-1 |
| Increased expression of “peripheral-type” benzodiazepine receptors |
| Alzheimer type II astrocytosis |
| Effects on the glutamate neurotransmitter system |
| Direct postsynaptic effects |
| Impaired neuron-astrocytic trafficking of glutamate |
| Inhibition of glutamate uptake |
| Altered glutamate receptors |
| Effects mediated by the formation of glutamine in brain |
| Cytotoxic brain edema |
| Increased uptake of aromatic amino acids |
| Other effects |
| Stimulation of L-arginine uptake and neuronal nitric oxide synthase (nNOS) expression |

Data from Ref. [70]
Cardiovascular Dysfunction

Patients with ALF are characterized by hypotension and tachycardia. The basis for this observation is vasodilatation in the periphery that results in relative hypovolemia, hypotension, and high output failure. Factors that contribute to this dysregulation are capillary leakage, low osmotic pressure, and systemic inflammatory response syndrome (SIRS). Persistent hypotension (mean arterial pressure, MAP <60 mmHg) should prompt volume substitution (normal saline or balanced electrolyte solutions) and vasopressor therapy, primarily noradrenalin [83]. In refractory shock, vasopressin should be administered where necessary in combination with hydrocortisone 300 mg I.V. daily as adrenal insufficiency may occur in a substantial number of patients with ALF [84].

Some patients with ALF may suffer from hypertension. This problem may arise, especially in patients with hepatic encephalopathy grade IV, and typically occurs when cerebral edema is evolving.

Infection

Infection and thus sepsis are major problems in patients with ALF. Patients with a long stay in the ICU have a very high risk in particular, and this may actually be the ultimate reason for death [85]. Studies from the King’s College Hospital group clearly indicated that monitoring by daily cultures (sputum, urine, and blood) identifies bacteria in up to 90% and fungal infections in around 30% of the patients [86, 87]. Frequently, the classical signs (fever, leukocytosis, and biochemical parameters, such as c-reactive protein and procalcitonin) in patients with ALF are not directly correlated with infection or are absent. The sites of the body with the most common infections are the lung, the urinary tract, and the blood (Fig. 29.7).

If antibiotic or antifungal treatment is necessary in these patients, the potential of further liver injury caused by antibiotic drugs should be considered. The basic principles are regular microbiological sampling and early antibiotic or antifungal medication, but no prophylactic antibiotics.

Besides the increased risk of patients being managed in ICU, additional factors contribute to the higher risk of infections in patients with ALF, namely, defects in the immunological defense mechanisms (complement, Kupffer cell function, polymorphonuclear cell function, and cell-mediated immune response). The liver is the main source of complement (e.g., C3 and C5) production. As a consequence of lower complement levels, the activity of polymorphonuclear leukocytes and complement-mediated opsonization is reduced.

Therefore, phagocytosis and killing of polymorphonuclear cells are inhibited in patients with ALF. Through the portal circulation, bacterial toxins are regularly brought to the liver tissue that are cleared by the resident Kupffer cells of the liver. In ALF, there is a correlation between hepatic damage and Kupffer cell dysfunction. Additionally, Kupffer cells are a major source of cytokines, and their dysregulation also contributes to impaired immune response. Defective lymphocyte function has been attributed to impaired interleukin-2 (IL-2) production in these patients. Thus, the defect in immune response can be explained on different levels of the immune system [3, 86].

Pulmonary Complications

Pulmonary complications are frequent [88]. Different mechanisms contribute to this observation. Up to 50% of the patients have infections, especially following intubation and subsequent mechanical ventilation [89]. The possible consequent capillary leakage can result in an ARDS-like syndrome that is further augmented by the infusion of albumin, fresh frozen plasma, and coagulation factors.

Besides these local mechanisms, systemic causes, as a result of liver failure, also lead to intrapulmonary vasodilatation and pulmonary arteriovenous shunting, which further increase the risk of hypoxic complications and deteriorate peripheral tissue oxygenation [90].

Renal Failure

Renal failure with oligo- and anuria is observed in up to 70% of patients with ALF and requires renal replacement therapy in 30% of cases [91]. In acetaminophen and amanita poisoning, as well as halothane toxicity, direct toxic effects additionally contribute to kidney failure.

The association between liver failure and kidney failure is functional and known as hepatorenal syndrome.
Pathophysiologically, the syndrome is characterized by a contraction of the vessels with a distinctively reduced renal perfusion. At this stage, renal dysfunction is potentially reversible. In the further course of ALF, which is typically characterized by progressive shock, tubular necrosis can occur, which potentially results in terminal renal failure.

Additional severe complications in patients with hepatorenal syndrome, such as long periods of hypotension or sepsis, have a fatal effect on kidney function and significantly reduce the prognosis of patients with fulminant hepatic failure [92].

As systemic inflammatory response syndrome (SIRS) has been identified as an independent predictor of renal dysfunction in patients with non-acetaminophen-induced ALF, SIRS has been suggested to be functionally linked to the development of renal dysfunction in patients with non-acetaminophen-induced ALF, but not in patients with acetaminophen-induced ALF [93]. Renal replacement therapy, mostly in the form of continuous veno-venous hemofiltration (CVVH), should be initiated early in patients with oliguria, volume overload, or clinically significant hyperammonemia (NH₃ >150 μmol/l).

Aside from renal replacement therapy, plasmapheresis, which is another extracorporeal procedure, is also a promising measure in ALF. A prospective multicenter study has investigated the effects of high-volume plasma exchange in ALF. About 182 patients received either standard treatment or treatment with complete plasma exchange with FFP for 3 days. A beneficial effect for plasmapheresis-treated patients who did not receive or could not receive liver transplantation was demonstrated, whereas patients receiving (or being listed for) liver transplantation did not significantly benefit from plasmapheresis [94].

Artificial liver assist devices, such as the molecular absorbent and recirculating system (MARS®) and the Prometheus® system, can improve HE but have no mortality benefit in ALF [95]. Their use should currently be limited to clinical trials.

### Metabolic Complications

The liver is essential for several metabolic functions. Two particular problems are frequently encountered in patients with ALF: hypoglycemia and acid-base disturbances.

Different mechanisms lead to hypoglycemia during ALF. The damaged liver loses its capacity to mobilize glycogen stores and to perform gluconeogenesis. Additionally, the liver is the major site of insulin metabolism, and the disintegration of insulin, which is consequently reduced, results in elevated insulin serum levels. All three mechanisms contribute to hypoglycemia, and this may also aggravate mental status. In terms of treatment, it might be important to differentiate hypoglycemia from hepatic encephalopathy as possible causes for disturbed mental status at certain stages. In ALF, glucose serum levels should be targeted at 140 mg/dl by glucose infusions [67].

Both acidosis and alkalosis might be present. Metabolic alkalosis is most frequent, as urea synthesis in the liver is impaired, which results in the accumulation of the two precursor substrates, bicarbonate and ammonium. Alkalosis is associated with hypokalemia, which is further aggravated by high sodium reabsorption in patients with ALF.

Acidosis, with a high anion gap, occurs in up to 30% of patients with acetaminopen poisoning and evolving acetaminophen-dependent ALF. In patients with a different etiology, acidosis is evident in only 5%. In those cases, acidosis is caused on the one hand by accumulation of lactate due to impaired microcirculation and resulting tissue hypoxia, and on the other hand by the inability of the injured liver to metabolize lactate. Early renal replacement therapy should be initiated in both, the event of severe metabolic acidosis and refractory hyperlactatemia.

### Coagulation Disorders

Bleeding complications in patients with ALF are uncommon, occur in approximately 10% of cases, and are usually clinically not significant [96]. Patients with ALF exhibit increased INR and various degrees of thrombocytopenia, depending on the extent of inflammation (e.g., SIRS/sepsis).

In ALF, these deficits in hemostasis are counterbalanced by compensatory mechanisms, such as hypersecretion of clotting factor VIII and von Willebrand factor by endothelium. Conversely, factor VIII might compensate the deficit of liver-derived coagulation factors and von Willebrand factor thrombocytopenia [97]. Interestingly, the development of thrombocytopenia in ALF is associated with the development of multiorgan failure and poor outcome [98]. Furthermore, procoagulant microparticles, as a result of systemic inflammation, might compensate for deficiencies in hemostasis.

In fact, the use of blood products (packed red blood cells, platelet concentrates, fresh frozen plasma [FFP], 4-factor prothrombin complex concentrate [PCC], and single coagulation factors) has been decreased during the past decades in the USA, whereas bleeding complications remained stable in approximately 10% of cases with ALF.

Without evidence of relevant bleeding, blood products should not be administered routinely. For signs of bleeding or thromboembolism, differentiated coagulation diagnostics (e.g., thromboelastography) and on-demand substitution is indicated [67].
Pathophysiological Aspects of ALF

ALF occurs when the extent of hepatocyte death exceeds the regenerative capacity of the liver. Mainly two different mechanisms of liver cell death can be differentiated: (1) direct cellular damage and activation of cell signaling cascade pathways, resulting in the disturbance of intracellular homeostasis, and (2) innate and adaptive immune responses leading to immune-mediated liver injury.

Similar to sepsis, patients with ALF commonly exhibit immune paralysis with characteristic features of systemic inflammation and cellular immune depression contributing to severe extrahepatic complications, such as multiple organ failure [85, 99]. In this context, cytokines exert crucial pathophysiological functions in ALF, comprising hepatocellular death, extrahepatic complications, and hepatocellular regeneration.

Dysregulation of the Cytokine Network in ALF

In the last years, it has become obvious that there is a dysregulation of cytokine expression during ALF in humans. For example, it has been shown that mediators of the acute phase response – IL-6 and tumor necrosis factor (TNF) – are strongly elevated in the liver and serum of ALF patients. The meaning of this observation becomes more evident through the development of animal models whereby the role of each of the molecule can be more clearly defined. As there is evidence that several cytokines might be involved in the pathogenesis of ALF, all the different aspects cannot be covered in this review. Here, we focus on two cytokines, TNF and IL-6.

IL-6/gp130-Dependent Signals

IL-6 interacts on the cell surface with the IL-6 receptor (gp80). This complex associates with two gp130 molecules, resulting in the activation of Janus kinases and in turn in the phosphorylation of tyrosines at the intracellular part of gp130. After phosphorylation of tyrosines, the ras/map kinase pathways and transcription factors Stat1 and Stat3 become activated [100]. In hepatocytes, IL-6 is one of the main inducers of the acute phase response, and in recent years, it has become evident that IL-6 also contributes to the regulation of additional pathophysiological conditions in the liver [101, 102].

One of the simplest models to study the loss of liver tissue is the removal of two-thirds of the liver by surgical resection. This model has been applied mainly in rodents (e.g., rat and mouse), and after 1–2 weeks, the liver tissue has been restored by hepatocyte proliferation. In recent years, it has become obvious that IL-6 and TNF are involved in the restoration of liver mass [103], as it...
has been observed that liver regeneration was impaired in IL-6 and TNF receptor 1 (TNF-R1) knockout mice after two-thirds hepatectomy. The defect in regeneration in both knockout strains could be restored through IL-6 stimulation [104, 105]. The model of how IL-6 and TNF may work in concert during liver regeneration following partial hepatectomy is presented in Fig. 29.8.

In humans suffering from ALF, the IL-6 serum levels are high, and in the liver infiltrating cells express tremendous (10-fold higher compared with controls) amounts of IL-6 [101, 102, 106]. In animal models of ALF, the IL-6 serum levels are also greatly increased [107], and treatment with a hyper-IL-6 designer molecule reduces liver cell damage in several animal models [108, 109]. IL-6 plays a protective role for hepatocytes, not only during liver regeneration but also during ALF; cDNA arrays further demonstrate that IL-6 activates antiapoptotic pathways, e.g., Bcl-xl, in hepatocytes [110, 111].

Most IL-6 data in animal models show that gp130-dependent pathways in hepatocytes activate protective mechanisms [101, 102], and in humans, it is also likely that IL-6 renders hepatocytes more resistant. Therefore, it might be promising to modulate IL-6/gp130-dependent pathways in humans during ALF as a potential therapeutic approach.

**TNF-Dependent Pathways**

TNF belongs to a family of several known Fas (CD95) and TNF receptor apoptosis-inducing ligands (TRAIL). There is also evidence of an involvement in the pathogenesis of fulminant hepatic failure. At present, the role of TNF has been studied in more detail in both human and animal models.

TNF binds to two receptors, TNF-R1 and TNF-R2, on the cell surface. After ligand binding, the intracellular domains of the receptors interact with adapter molecules that activate different pathways (see Fig. 29.8). In the case of TNF-R1, first the molecule TNF-R-associated death domain (TRADD) and then additional molecules bind to activate the caspase cascade either via Fas-associated death domain (FADD) or via TNF-associated factor/receptor-interacting protein (TRAF/RIP) jun kinase (JNK) and nuclear factor-kB (NF-kB) (see Fig. 29.8) [112].

It has become evident that besides inducing apoptosis, TNF can also trigger necrosis. Therefore, TNF and its family members seem to be essential mediators of cell death during ALF. In humans, it has been shown that TNF serum levels correlate with the prognosis in ALF patients [106]. In animal models, blocking experiments using anti-TNF attenuates liver failure, and therefore, it is obvious that TNF plays a central role in the pathogenesis of ALF. However, further studies indicated that TNF has no uniform role in the different models. Depending on the model, the TNF-dependent effect might be related to a different cell in the liver or another intracellular pathway. Three models of ALF and the role of TNF will be discussed.

**Endotoxin/Galactosamine Model**

During LPS/galactosamine (GaIN)-induced liver injury, TNF induces the transcription of several pro-inflammatory genes, e.g., chemokines, nitric oxide, and adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P-selectin [113–115]. These changes in the liver are essential for triggering the extravasation of neutrophils into the liver parenchyma, which results in cytotoxic liver cell damage. During this scenario, a stepwise cascade has been described, which consists of three events: (1) sequestration of neutrophils in the liver vasculature, (2) transendothelial migration, and (3) adherence-dependent cytotoxicity against hepatocytes [116].

Therefore, in the LPS/GaIN, model TNF obviously triggers an inflammatory mechanism mediated via NF-kB that results in liver cell damage. In this model, not only parenchymal but also non-parenchymal cells are involved in this process.

**Galactosamine/TNF Model**

The administration of GaIN and TNF triggers apoptosis of hepatocytes *in vivo* and *in vitro*. The essential role of TNF-R1 in this model has been demonstrated by TNF-R1 knockout mice that are resistant against GaIN/TNF treatment [117]. GaIN directly inhibits transcription and thus synthesis of antiapoptotic signals. Therefore, in this model, the FADD-dependent pathway leading to apoptosis is the essential step in ultimately inducing liver cell damage. In contrast, the NF-kB and JNK pathways do not seem to be involved in the pathogenesis of liver damage, and also, nonparenchymal cells play no role. In this model, simple administration of an adenoviral construct expressing a dominant molecule blocking the FADD pathway is protective [106]. These data indicate that the caspase cascade activated by TNF might be a relevant target during ALF.
Concanavalin A (ConA) is a lectin with high affinity towards the hepatic sinus [118]. Accumulation of ConA in the hepatic sinus results in the activation of liver natural killer T (NKT) cells, i.e., NK 1.1 CD4+ CD8- T cell receptor (TCR)αβ, and NK1.1. CD4+ CD8- TCR αβ, which are essential for triggering the early phase of ConA-induced liver injury [119, 120]. Consecutively, CD4-positive and polymophonuclear cells are attracted to the hepatic sinus and trigger an increase in cytokines, such as TNF, IL-2, IFN-γ, IL-6, granulocyte macrophage colony-stimulation factor (GM-CSF), and IL-1 [58]. TNF-α and IFN-γ have direct implications for the induction of liver cell injury, whereas anti-TNF-α and anti-IFN-γ antibodies have protective effects in ConA-induced liver injury [121, 122] and IFN-α/− and TNF-α mice are resistant to ConA-induced liver cell damage.

Until now, a stepwise process of liver damage, as shown for the endotoxin/LPS model, could not be defined for the ConA model. Adhesion molecules, such as ICAM-1 or VCAM-1, seem to play a minor role. Mice pretreated with antibodies against both adhesion molecules or ICAM-1 knockout mice still undergo liver cell injury [123].

Recently, it has been shown that hepatoocyte-specific caspase-8 knockout mice are more susceptible to ConA-induced liver injury [124]. These results show that during ConA-induced liver injury, necrosis is the more prevalent form of cell death. Therefore, the ConA model is especially helpful to better define this form of hepatocyte injury in vivo.

Apoptosis and Necrosis in ALF

Apoptosis – the programmed form of cell death – is inevitable to maintain the balance of cell proliferation and elimination of injured cells. Caspase proteases are involved in the initiation, execution, and regulation of apoptotic pathways. Effector caspases (e.g., caspase-2, -6, -7) cleave various cellular proteins (e.g., cytokeratin-18) [125], and initiator caspases (e.g., caspase-8, -9, -10) exhibit regulatory functions by activating the downstream effector caspases [126]. The major signaling routes for caspase activation are the extrinsic death receptor and the intrinsic mitochondrial pathway [127] (see Fig. 29.6).

Death receptors are transmembrane proteins that consist of the following domains: (1) extracellular ligand-interacting domain, (2) transmembrane domain, and (3) intracellular death domain. Typically involved in ALF are death receptors CD95 (Fas), tumor necrosis factor receptor 1 (TNF-R1), and tumor necrosis factor-related apoptosis inducing ligand receptors 1 and 2 (TRAIL-R) and death receptors 3 and 6. Binding of death ligands such as TRAIL, CD95L, and tumor necrosis factor to their specific receptors leads to the recruitment of the adapter protein FADD and caspase-8 into death-inducing signaling complex (DISC), wherein caspase-8 is activated [128]. In most cells and hepatocytes, respectively, only low amounts of caspase-8 are activated in the DISC, which is not effectual for cell death. In order to exert cell death, the extrinsic receptor pathway has to be amplified by the intrinsic mitochondrial apoptotic pathway through the caspase-8-effected cleavage of Bid (a pro-apoptotic Bcl-2 family protein). Subsequently, together with the Bcl-2 family members Bak und Bax, the release of pro-apoptotic mediators from the mitochondrion is initiated [129].

ALF, induced by agonistic CD95 antibody, could be abolished by silencing of CD95- or caspase-8-protected mice [130, 131]. Conversely, CD95 and caspase-8 are involved in liver regeneration by inducing the differentiation of stellate cells and other non-parenchymal liver cells [132, 133]. TNF-α plays a key role in liver regeneration by activating NF-kB, which exerts antiapoptotic functions in the liver [134].

Necrosis is mediated by opening of the mitochondrial membrane permeability transition (MPT) pore, leading to the disruption of ATP formation and finally resulting in mitochondrial swelling and rupture of the outer mitochondrial membrane. Interestingly, it has been recently shown that TNF can also induce controlled necrosis. Therefore, necrosis is now also considered a programmed form of cell death, which is initiated by RIP1/RIP3 activation. Additionally, massive ATP depletion, formation of reactive oxygen species (ROS), activation of non-apoptotic proteases, and strongly increased intracellular calcium concentrations – aggravating ATP deficiency by loss of mitochondrial membrane potential – contribute to necrosis. As loss of ATP leads to necrosis and apoptosis is ATP-dependent, the intracellular amount of ATP itself might determine the way of cell death, either by apoptosis or by necrosis [135, 136]. Necrosis is associated with inflammation, as necrotic cell rupture induces an inflammatory response owing to the release of intracellular components, including the M65 form of cytokeratin-18 (CK-18), whereas apoptotic cells are rapidly cleared by phagocytic cells, thereby preventing the release of intracellular contents.

Cytokeratin-18 as a Prognostic Biomarker in ALF

CK-18 is a filament protein cleaved by caspases into specific fragments, which can be measured in the serum using the M30 ELISA (Fig. 29.9).

CK-18 levels at the time of admission have been demonstrated to be predictive of mortality in patients with ALF, with a prognostic impact comparable with the model for end-stage liver disease (MELD). Additionally, a modified MELD score where uncleaved necrotic CK-18 (M65 ELISA) substi-
tuted for bilirubin predicted significantly better prognosis of ALF patients compared with the current MELD score [137].

The observation that ALF patients who die or require transplantation exhibited increased serum levels of total CK-18, but the reduced levels of caspase cleaved fragments indicate that necrosis, not apoptosis, is the more prominent cell death mode in these most critically ill ALF patients [138]. In line with this, patients with acetaminophen-induced liver injury, where necrosis is the predominant cell death mode, exhibited higher levels of total CK-18 than caspase cleaved CK-18.

**Intestinal Microbiome and Acute Liver Failure**

The link between intestinal dysbiosis and chronic liver disease is well described by numerous studies [139]. Moreover, the intestinal-microbiota-liver axis has been proposed as a promising target to prevent the progression of chronic liver disease [140]. Nevertheless, little is known about a potential functional link of gut-liver interaction during ALF. Recently, interesting data of an animal model with wild-type (WT) and dysbiotic Nlrp6−/− mice and liver injury induced by acetaminophen (APAP) or lipopolysaccharide (LPS) have been presented [141]. Liver injury was studied based on liver functions tests, histology, flow cytometry immunophenotyping (FACS), and 16S rRNA-based microbiota profiling.

Interestingly, dysbiotic Nlrp6−/− mice exhibited significantly increased liver injury, as assessed by the extent of hepatic inflammation and necrosis compared with WT controls. Enhanced liver damage in Nlrp6−/− mice was associated with markedly increased infiltration of Ly6Chi monocyte-derived macrophages (MoMFs). As a potentially protective response to hepatic injury, WT mice exhibited a shift in microbiota composition and an expansion of colonic mucus layers, whereas this effect was absent in Nlrp6−/− mice. Fecal microbiota transfer (FMT) from Nlrp6−/− mice into WT mice aggravated liver injury upon APAP treatment in WT mice with a Ly6Chi inflammatory phenotype. These data reveal novel, so far unknown functions of intestinal microbiota during ALF and identify intestinal dysbiosis as a driver of ALF severity (Fig. 29.10). Future clinical studies should investigate the intestinal microbiome as a promising therapeutic target in ALF.

**Translation of Experimental Data Into Therapeutic Approaches in Humans**

The current data in animal models and humans indicate that TNF plays a significant role in the pathogenesis of ALF. However, as demonstrated for the three animal models discussed, depending on the pathogenesis, the intracellular pathways activated by TNF could have opposing effects. The mode of liver cell death in ALF is still controversial. Induction of apoptosis or necrosis of hepatic cells potentially depends on the etiology and the duration and extent of liver injury. Severe liver damage causes oxidative stress and concomitant depletion of ATP, resulting in necrosis. Conversely, sufficient cellular ATP stores are essential for the execution of apoptosis. Necrosis as a consequence of severe hepatic injury is associated with an unfavorable prognosis.
Potentially, the differentiation of necrosis and apoptosis might enable the early identification of patients requiring transplantation. The identification of the molecular cell death mechanisms might offer new therapeutic perspectives for ALF. Reduction of cellular death without inhibition of the hepatic regenerative capacity seems to be the main goal for new therapeutic interventions. Whereas extreme liver injury results in necrosis, milder injury leads to apoptosis. Potentially, inhibition of apoptosis by caspase inhibitors can prevent liver cell death but can also possibly change only the cell death mode from apoptosis to necrosis. Considering the therapeutic use of caspase inhibitors to prevent apoptosis, the involvement of caspases in liver regeneration must not be ignored, as this might lead to potential severe adverse effects. Therefore, further studies are required to better understand the molecular mechanisms that determine the mode of cell death during ALF.

In mouse models, the administration of cyclooxygenase (COX) inhibitors resulted in decreased oxidative stress and reduced hepatic necrosis [142]. Therefore, COX inhibitors could be further investigated as potential agents to prevent ALF.

Another promising novel target in acetaminophen-induced ALF is cyclophilin A. Cyclophilin A is an intracellular protein that is pro-inflammatory when released by cells. In an animal model of acetaminophen-induced liver injury, it has been demonstrated that cyclophilin A acts as a DAMP to mediate acetaminophen toxicity and that experimental inhibition of cyclophilin A ameliorates acetaminophen-induced liver injury [143].

Recent data hint at so far unknown functions of intestinal microbiota during ALF. Intestinal dysbiosis of Nlrp6−/− mice was transferrable to healthy wild-type controls by fecal microbiota transfer which led to pro-inflammatory Ly6Chi macrophage polarization and finally resulted in the aggravation of hepatic injury.

**Concluding Remarks and Open Questions**

ALF is characterized by the sudden onset of liver failure in patients without evidence of chronic liver disease, by which ALF is differentiated from end-stage chronic liver disease. According to the time between the first symptoms and encephalopathy, ALF is divided into three subgroups: hyperacute, acute, and subacute. The prognosis of ALF patients is determined by the metabolic situation resulting from the loss of liver cell mass, the release of mediators and toxic metabolites from injured liver tissue, and the capacity of the remaining vital hepatocytes to restore functional liver mass.

Suicidal acetaminophen ingestion is the most frequent cause of drug-induced liver failure worldwide, with approximately 500 deaths a year in the USA. Other important mechanisms are viral hepatitis and cardiovascular and metabolic disorders.

ALF leads to multiorgan failure, especially to cerebral edema and encephalopathy. Owing to the diminished liver function, higher rates of infections and coagulation disorders are observed. Cerebral edema, infections, and renal failure are important clinical complications limiting survival. For risk stratification in patients with ALF and subsequent hepatic encephalopathy, serum ammonia levels can be used. Advanced cerebral dysfunction is expected at serum ammonia levels of 124 μmol/l or higher.
Cardiovascular dysfunction is characterized by peripheral vasodilatation that results in relative hypovolemia, hypotension, and high-output failure. Capillary leakage and high-volume therapy can lead to an ARDS-like syndrome and cause hypoxic complications. Prothrombin time is a useful parameter for assessing the extent of the remaining liver function.

Intensive care therapy is crucial for patients with ALF for managing multiorgan failure, and mild hypothermia to reduce cerebral edema should be considered. Further research and controlled clinical studies are required to evaluate the importance of hypothermia.

The mode of liver cell death which is predominantly induced in ALF (apoptosis or necrosis) is potentially determined by the underlying etiology, the duration of the disease, and the extent of liver injury. Severe liver injury leads to oxidative stress and depletion of ATP stores favoring necrosis, whereas sufficient cellular ATP resources are required for the execution of apoptosis. As necrosis is associated with an inferior outcome as compared with apoptotic cell death, the discrimination of the cell death mode in ALF might be a prognostic tool to instantly identify patients requiring transplantation. Moreover, the molecular cell death mechanisms in ALF are promising targets for future research aiming at reducing hepatocellular death without inhibiting liver regeneration.

The potential functional link of gut-liver interaction during ALF, where dysbiosis has been recently identified as potential driver of ALF severity, might be a promising novel therapeutic target and future studies should aim at further investigating the significance of the intestinal microbiome in ALF.

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