Acute Hepatitis E Virus Superinfection on Top of Liver Cirrhosis

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Abstract

Despite acute HEV infection is self limiting acute infection ,it could be a cause of ACLF in patients with chronic liver disease. Thus identification role of HEV superinfection in deterioration of liver cirrhosis with early treatment could save life of those patients.

KeyWords: Hepatitis E Virus, liver failure, superinfection.

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Introduction.

The hepatitis E virus (HEV) is **a** common cause of acute viral hepatitis worldwide and belongs to the Hepeviridaefamily (**1).**

The HEV is a small non-enveloped virus,about 27-34 nm in diameter single- stranded (RNA) genome. The HEV genome contain discontinuous regions named open reading frames (ORF), ORF1encodes nonstructural (functional) proteins (e.g., RNA-dependent RNA polymerase)**(2)**

ORF2 encodes the viral capsid protein, the capsid protein encoded by ORF2 is highly Table (1) showing HEV genotypes **(5)** immunogenic, and antibodies against this protein have neutralizing and protective Features. So,the capsid protein suitable target for vaccine development against HEV. ORF3 encodes functional ion channel that has value in the release of viral particles **(3)**.

ORF4 recently discovered is unique for HEV genotype 1 (HEV1) and plays a critical role in the proper functioning of HEV RN polymerase**(4).** There are eight HEV genotypes, HEV1, HEV2, HEV3, and HEV4 are able to infect humans .

HEV genotype	Area	Sources of infection	Comment
GT 1	Tropical developing countries of Asia and Africa	Contaminated drinking water	No zoonotic relevance No chronic infections described
GT 2	Tropical countries of Africa or Mexico/ Central America	Contaminated drinking water	No zoonotic relevance No chronic infections described
GT 3	Industrialized nations, worldwide distribution, autochthonous in Europe, North and South America, Australia and large parts of Asia	Foodborne zoonosis → Swine → Deer → Cats → Rats → Rabbits → Mongooses → Cows → Cow's milk → Sheep → Strawberries → Vegetables → Blood products	Chronic HEV infections described in several cohorts of immunosuppressed patients
GT 4	Mainly in Asia, recently single cases in Europe	Foodborne zoonosis → Swine	Chronic HEV infections described in single immunosuppressed patients
GT 5	Japan	Wild boar	Relevance for humans still unclear
GT 6	Japan	Wild boar	Relevance for humans still unclear
GT 7	Middle East	Dromedary camels (one-humped camels)	Chronic infection in a liver transplant recipient who regularly consumed camel meat and milk
GT 8	Middle East	Bactrian camels (two-humped camels)	Relevance for humans still unclear

Humans are the main reservoir of HEV1 and HEV2, and any transmission from animals to humans for HEV1 and HEV2 has not yet been reported. **(6)**

The person-to-person transmission of HEV1 and HEV2 is infrequent in both sporadic and epidemic settings, whereas vertical transmission from mother to fetus during pregnancy is well defined **(7)**.

Moreover, HEV1 transmission by blood transfusion also reported. HEV3 and HEV4 transmission is zoonotic caused by close contact with infected animals or the consumption of contaminated food products (most commonly raw or undercooked meat (**8).**

HEV transmissioncan occur from a transplanted organ **(9)**. HEV5 and HEV6 have only been reported in wild boars and are not associated with infections in human beings **(10).** HEV7 and HEV8 have been found in camels **(11)**

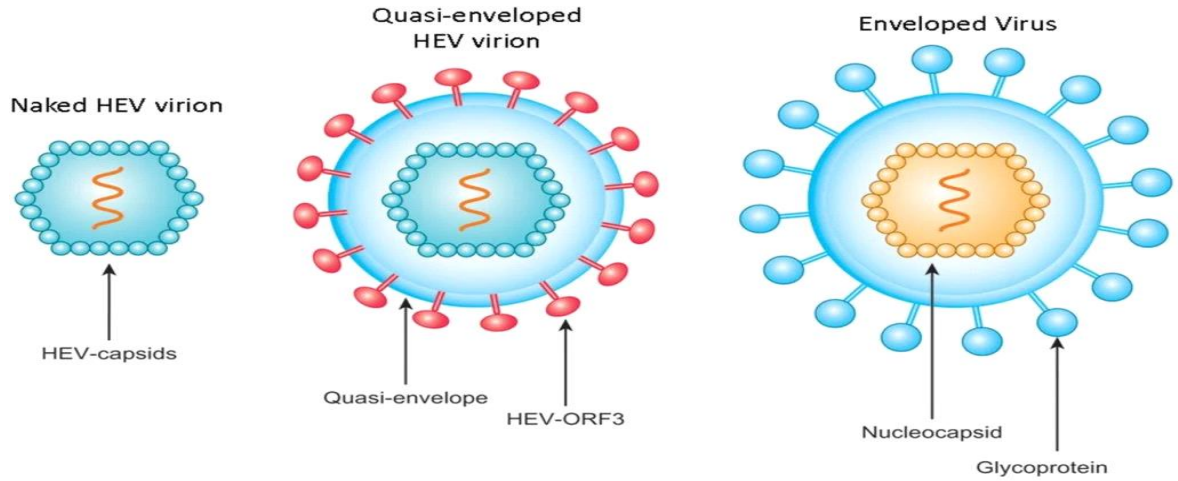

Fig (1): Structure of HEV (12).

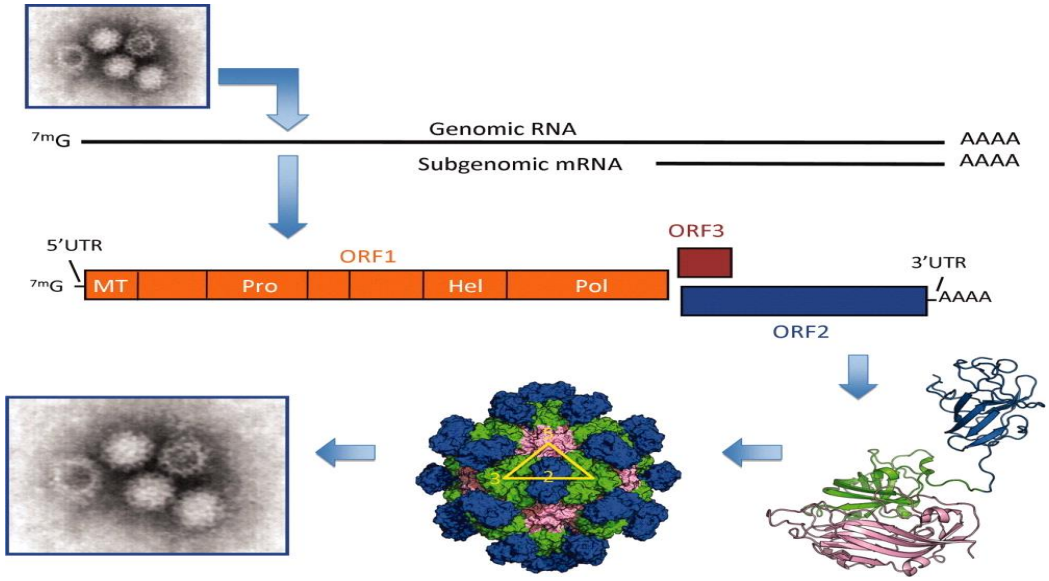

Fig (2): HEV and its genome. HEV particles contain a positive-sense genomic RNA of ~7.2 kb (kilobases) that is capped and polyadenylated and carries short 5′ and 3′ untranslated regions (UTRs). During genome replication, a subgenomic RNA of ~2 kb is also produced. The genomic RNA carries three open reading frames (orfs) that encode the nonstructural ORF1 (orange), capsid ORF2 (blue), and regulatory ORF3 (brown) proteins. The ORF1 polyprotein carries various biochemical domains: methyltransferase (MT), protease (Pro), helicase (Hel), and RNA-dependent RNA polymerase (Pol). The ORF2 protein monomer contains three domains (shown in pink, green, and blue) that make up different structural elements on the HEV particles. The icosahedral 2-, 3-, and 5-fold symmetry axes are indicated. (**13)**

Although HEV1 and HEV2 usually lead to self-limiting acute viral hepatitis, HEV1- and HEV2-related infections still have a substantial burden on public health in lowincome countries. According to the mathematical model developed in 2005, these genotypes were associated with 20.1 million annual new infections in Asia and Africa with 3.4 million symptomatic hepatitis E cases, 70000 fatalities attributed to acute liver failure, and 3000 stillbirth **(14)**

- **Clinical manifestations of HEV**
  - **Acute HEV infections**

Acute HEV infection is relatively asymptomatic , However acute icteric hepatitis is seen in almost 5%-30% of patients .

Prodromal phase characterized by Malaise, fever, body aches, nausea, and vomiting

followed by the icteric phase lasting approximately 1 week manifested by dark urine and jaundice Then, the convalescent phase leading to resolution of icteric symptoms **(15**).

Generally, HEV1 and HEV2 cause more severe acute hepatitis presentation than HEV3 and HEV4**(16)**

HEV1 infection in a pregnant woman (particularly at the third trimester) carries high risk ofmaternal morbidity and mortality. up to 20% and may lead to eclampsia, hemorrhagic complications, and liver failure **(17)**

The newborns have a risk of maternal–fetal transmission and clinicalmanifestations such as hypoglycemia, hepatitis, and neonatal death **(18**).

- **ACLF(Acute on top of chronic liver disease)**

ACLFdefined as acute worsening of the liver function with clinical complications such as the development or deterioration of ascites, hepatic encephalopathy and coagulopathy in patients with chronic liver disease.

- **Chronic HEV infections in immunoco-mpromised patients**

Immunocompromised patients cannot clearHEV and may develop chronic hepatitis and cirrhosis if infected by HEV3 and HEV4 **(19).**

In contrast, chronic HEV infection has not been observed in cases infected with HEV1 and HEV2 until now **(20**). Chronic HEV infection insolid organ transplant recipients documented in patients who are viremic for more than 3 mo after the onset of HEV infection **(21).**

Diagnosed byperforming (HEV RNA PCR) in all patients for the detection of chronic HEV infection since both anti-HEV IgG and IgM may remain negative under immunocompromised status **(22)**

Although fatigue is the main symptom in chronic HEV infection, most patients have no symptom and only mild elevations in liver enzymes **(22)**

Chronic HEV infections may lead to structural injuries in the liver including nodules,fibrotic remodeling, and subsequent cirrhosis **(23)**

The risk of the development of chronic HEV infection after HEV3 exposure is related to previous tacrolimus use and low lymphocyte count, not related to the HEV load **(24).**

- **Extrahepatic manifestations**

HEV infections not only affect the liver but may also include other organ systems leading to Guillain-Barre syndrome (GBS), neuralgic amyotrophy (NA), lymphoma, pancreatitis, thrombocytopenia, viral meningitis, thyroiditis, myocarditis, cryoglobulinemia, glomerulonephritis, Henoch-Schönlein purpura, and myasthenia gravis.

The accurateunderlying pathophysiological mechanisms have not yet been detected but immune-mediated reactions and direct viral (cytopathic) tissue damage are the most mechanisms in extrahepatic manifestation **(25).**

- **Diagnosis:**

The incubation period of HEV infection is usually 2-6 weeks **(26)**

Acute HEV infection diagnosed by Anti-HEV IgM antibodies which lasting in serum for a short period of time (approximately 3-4 months), but sometimes it persists for a year**(27)**

Anti-HEV IgG antibody lasting for long duration and the exact duration not detected defintly**(28)**

ELISAis the most widely used serological method for the detection of anti-HEV IgG and IgM antibodies in the diagnosis of HEV infection . **(29)**

HEV RNA can be detected in the blood after 3 weeks of exposure and viral shedding lasts approximately 4-6 weeks in the stool**(29)**.

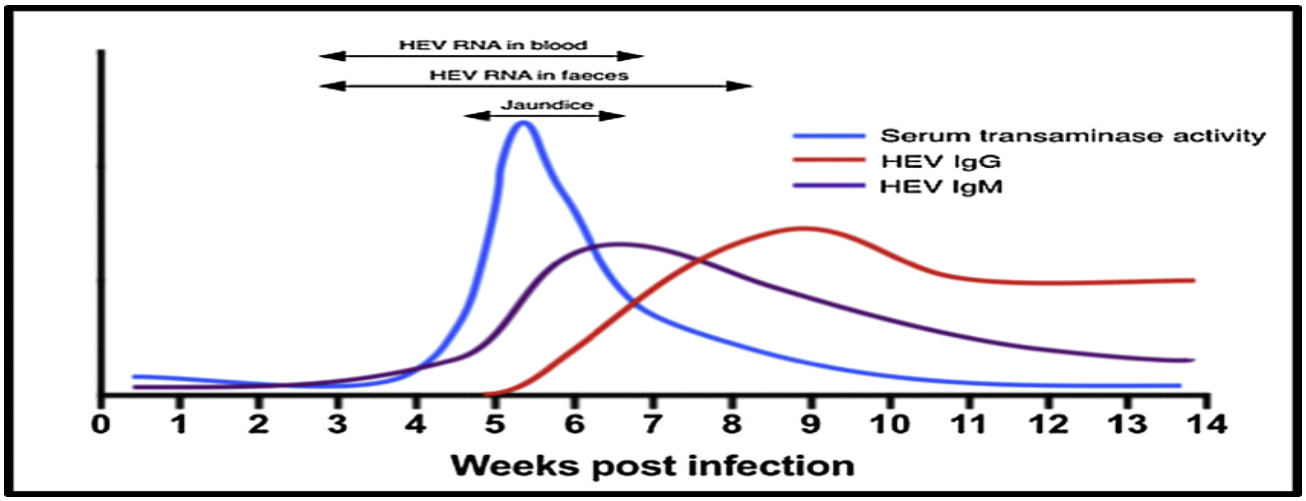

Fig (3): Graph demonstrating how HEV viral load varies with respect to development of jaundice and seroconversion(30)

- ***Treatment***
  - **Acute HEV infection** commonly cleared spontaneously and does not require any specific treatment.

However, acute HEV infection can progress to severe hepatitis and liver failure particularly in pregnant women and patients with underlying chronic liver diseases.

In severe cases like ACLF, rapid clearance of the HEV and normalization of liver enzymes were recorded with ribavirin treatment for 3 months at dose of 600 -1200 mg \day **(31)**

Corticosteroids were investigatedfor slowing down the rate of progression to liver failure in patients with fulminant hepatitis (***21).***

Ribavirin treatment iscontraindicated in pregnant patients because of the teratogenicity of ribavirin, **(32)**. Early liver transplantation should be considered (**33).** Therapeutic termination of pregnancy cannot be advised based on the current literature (***34)***

- **Treatment of chronic HEV infection;**

The main therapeutic approach for solid organ transplant recipients who have chronic HEV3 or HEV4 infection should be dose reduction of immunosuppressive medications, particularly those targeting T lymphocytes, this can be successful provides sustained viral clearance in up to one-third of patients **(31)**

Although pegylated interferon-alpha can be considered in liver transplant recipients for the treatment of chronic HEV infection **(35)**

Pegylated interferon isnot indicated in renal, pancreas, heart, and lung transplant patients because of enhanced immune response and the risk of rejection **(36)**.

Rribavirin remainthe treatment of choice in chronic HEV infections in many solid-organ transplant recipients, despite that its efficacy has not been accepted by randomized controlled trials.

In a multicenter retrospective study including 59 solid organ transplant recipients treated with ribavirin at a median dose of (600 _ 1200) mg/d for 3 months, the rate of sustained virologic response (undetectable HEV RNA in serum 6 mo after the completion of ribavirin treatment) was 78%. Additionally, one-third of patients with persistent HEV viremia at the end of the 3-mo treatment, achieved sustained virologic response after receiving ribavirin for a longer period **(14)**

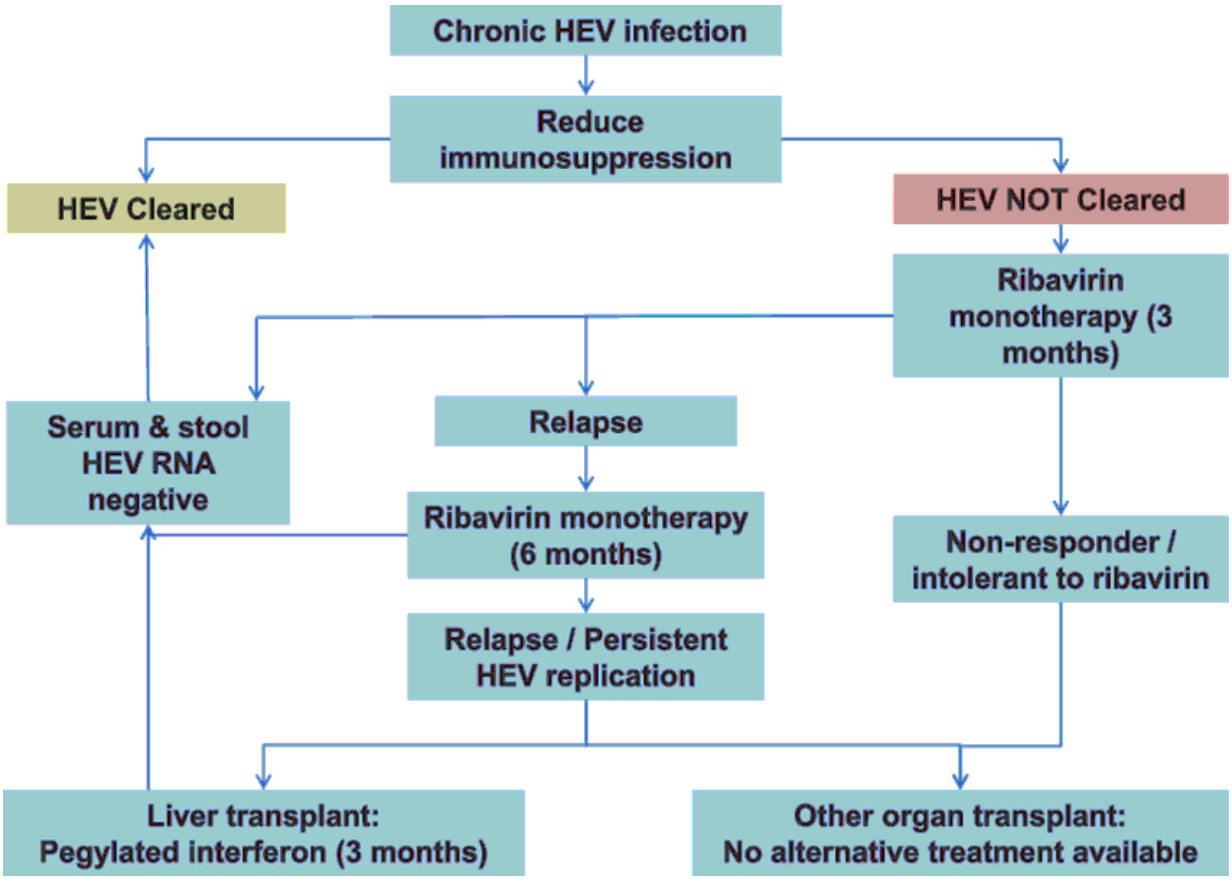

Fig (4): treatment of chronic HEV in immunocompromised (organ transplant) .(37)

Other Predictors of better response for ribavirinarehigher baseline lymphocyte count and haemoglobin level(i.e., not needing ribavirin dose reduction), Nevertheless, tolerability of ribavirin treatment is still a major concern .

The optimal duration of ribavirin therapy remains unclear, the 3-months treatment is the most widely used treatment duration for chronic HEV infections. At the completion of ribavirin treatment, the detection of HEV RNA in the stools of patients in whom HEV RNA was negative in the serum was reported as being associated with an increased riskofHEVviremia at follow-up **(38)**

Ribavirin inhibit HEV replication by depleting guanosine triphosphate pools, which probably inhibits inosine monophosphate dehydrogenase and prevents HEV RNA replication **(39)**

Ribavirin treatmentside effectsincluding skin reactions, dose- dependent hemolyticanemia, and dry cough. As patients with chronic HEV infection have some comorbidities resulting in impaired renal function or anemia, ribavirin doses should be adjusted cautiously in these patients **(40)**.

- **Treatment of extrahepatic complications**

The extrahepatic manifestations of HEV infections can be treated by either ribavirin or immune suppressive medications such as corticosteroids.

Extrahepatic manifestations are mainly explained by immunological mechanisms or the direct viral (cytopathic) effect of the HEV, Therefore, treatment (ribavirin or immunosuppressive drugs) should be chosen according to the main pathophysiologic mechanisms of extrahepatic manifestations.

TREATMENT OPTIONS FOR RIBAVIRIN-RESISTANT HEV INFECTIONS till now not approved still under trial

Sofosbuvir was tried as an option in the treatment of ribavirin-resistant HEV infections, However, a study involving 10 cases with chronic HEV infection reported only partial response and high rate of relapse with sofosbuvir monotherapy**(41)**

Moreover, it was administered as combination therapy with ribavirin in some ribavirin-resistant cases showed the clearance of the HEV with sofosbuvir/ribavirin combination therapy in patients with acute HEV infection. **(42)**

In contrast, other studies demonstrated that sofosbuvir/ribavirin combination therapy was not beneficialto gain sustainedvirologic response in chronic HEV infections observed in solid-organ transplant recipients and patients infected by the HIV **(43)**

Promising compound, 2'-C-methylguanosine, that suppressed the growth of HEV3 in cell cultures and showed in vitro synergistic interaction with ribavirin against the HEV. However, there is no study yet investigated efficacy and safety in animal models and human trials. **(44)**

Zinc can be a possible adjuvant therapy in ribavirin-resistant and/or relapsed HEV infections. Additionally, authors identified significantly lower serum zinc level in patients with chronic HEV infection than in the control group **(41)**

In vitro, the natural compound silvestrol has an inhibitory effect on HEV replication, Additionally, silvestrol -treated mice showed a rapid diminish in fecal concentrations of HEV RNA,However, it has not yet been tested on humans. **(45)**

NITD008 and GPC-N114 compounds were originally developed to treat the dengue virus and picornaviruses, respectively, These two novel antiviral candidates demonstrated a potent inhibitory effect against HEV replication without causing significant cellular cytotoxicity in cell cultures **(46)**

However, the antiviral efficacy and safety of thesecompounds are still unknown for HEV infections in humans.

- **VACCINE:**

In 2010, an HEV vaccine, based on a protein encoded by ORF 2 of an HEV1, was assessed in a phase 3 trial including more than 100000 participants from China **(47)**.

In this phase 3 trial, the long-term efficacy and safety of this vaccine was explored over more than 4 years in a vaccinated group (n = 56302 participants) in comparison with a control group (n = 56302 participants). The authors of this trial identified only 60 cases of hepatitis E, and seven of them belonged to the vaccinated group. Furthermore, no serious adverse events related to the vaccine were observed **(47)**

Because of the endemicity of HEV1 and HEV4 in China, the protective effect of this vaccine could be assumed for HEV1 and HEV4, but these findings cannot be illustratedfor HEV3 infections. That is why the National Institute of Health decided to perform a phase 1 trial to investigate the safety of this vaccine, and phase 2 and 3 trials will likely follow that trial. Therefore, the findings from these trials will demonstrate the safety and efficacy of HEV vaccine (Hecolin) in an HEV3 endemic region.

Vaccine yields sterilizing immunity, Another trial evaluated the immunogenicity and safety for an accelerated vaccination regimen (0, 7 and 21 days) and was safe , This dosage regimen can be recommended for travellers visiting an HEV-endemic regions or used during an HEV outbreak(**48).**

Additionally, an ongoing large trial is testing Hecolin in more than 20000 pregnant women in Bangladesh. The results of this study would be quite important to understand the effectiveness and safety of Hecolin in pregnant women who are under great risk of HEV1 infections.**(49).**

Acute on top of chronic liver failure

In 2009, the Asian Pacific Association for the Study of the Liver **(APASL)** established the first agreed-upon definition for ACLF: ‘‘acute liver damage manifested as jaundice (bilirubin ≥5mg/d) and coagulopathy (INR ≥1.5), complicated in the periodof 4 weeks with ascites or encephalopathy’’ in patients of chronic liver disease**.(50)**

- **ACALF types** according to the stage of the underlying chronic liver disease: **Type** ACLF (patients with chronic liver disease without cirrhosis); **Type B ACLF** (patients with compensated cirrhosis); and **Type C ACLF** (patients with decompensated cirrhosis)**(51).**

Table (2): Grades of ACALF (52)

ACLF grade[a]	Defining features[a]	Organ failure definitions[b]
0 (no ACLF)	No organ failure Single nonrenal organ failure + sCr < 1.5 mg/dl and no hepatic encephalopathy Single neurologic failure + sCr < 1.5 mg/dl	Neurologic: Hepatic encephalopathy grade III/IV[c] Circulatory: Requirement of vasopressor(s) Respiratory: PaO_2/FiO_2 of ≤200 or SpO_2/FiO_2 of ≤214 · mechanical ventilation Hepatic: Bilirubin ≥12 mg/dl Renal: sCr ≥ 2.0 mg/dl or renal replacement therapy Coagulation: INR ≥ 2.5
1	Single renal failure Single liver, coagulation, or respiratory failure + sCr 1.5–1.9 mg/dl and/or hepatic encephalopathy grade I/II[c] Single neurologic failure + sCr 1.5–1.9 mg/dl	
2	Two organ failures (any organ systems)	
3	Three or more organ failures (any organ systems)	

- **Pathophysiological Mechanims in ACLF:**

In general, bacterial infections, followed by active alcoholism and the reactivation of HBV are the most common precipitating factor. **(53).**

In Europe and the United States, bacterial infections and alcoholism are the most common identifiable factors, which represent around 30% and 20% of the cases of ACLF, respectively**.(52)**

In Asia, reactivation ofHBVfollowedby bacterial infections are the most common precipitating factors, with 36% and 30%, respectively. **(54)**

However, a precipitatingfactorwasnotidentifiedin significant numberof patients(up to around 20---40%) **(54)**

The precipitating factors can either **‘‘intrahepatic’’**, such as alcohol consumption, reactivation of HBV or acute hepatitis due to HAV or HEV, or **‘‘extrahepatic’’,** which are mainly bacterial infections or gastrointestinal bleeding, among others. **(54)**

• **Inducers of systemic inflammation:**

Systemic inflammation can be induced by the presence of pathogen-associated molecular patterns **(PAMPs)** and damage-associated molecular patterns **(DAMPs).PAMPs** expressed by microbes are unique molecular structures that are recognised by pattern-recognition receptors (**PRRs),**an example being Toll-like receptors **(TLRs**), which are expressed in innate myeloid cells (i.e., monocytes and neutrophils) and other cells of the innate immune system. **(55)**

PRR engagement drives intracellular signalling cascades, ultimately leading to transcription and synthesis of inflammatory mediators. A classicalexampleof these mechanisms is the engagement of TLR4 by lipopolysaccharide, **a PAMP** derived from the cell wall of gram-negative bacteria, resulting in the downstream transcription and activa- tion of multiple inflammatory mediators and cytokines**(56).**

High levels of circulating **PAMPs**, which are unrelated to bacterial infections but mostly related to translocation of bacterial products from the intestinal lumen may contribute to cases of ACLF without any identified precipitating disorder**.(57)**

These translocated **PAMPs** are the final result of intestinal bacterial overgrowth, increased permeability of the intestinal mucosa and impaired function of the intestinal innate immune system. **(58)**

Bacterial virulence factors can induce inflammation, not through their direct recognition by PRRs but through functional effects; for example ,toxins, induce a K+ efflux through the cell membrane that contributes to the activation of the NLRP3 (NLR family pyrin domain containing 3)inflammasome**.** Systemic inflammation can also occur in the absence of infection, this sterile inflammation is due to the release of circulating **DAMPs** by dying or damaged host cells that bind to and activate specific **PRRs**). **(59).**

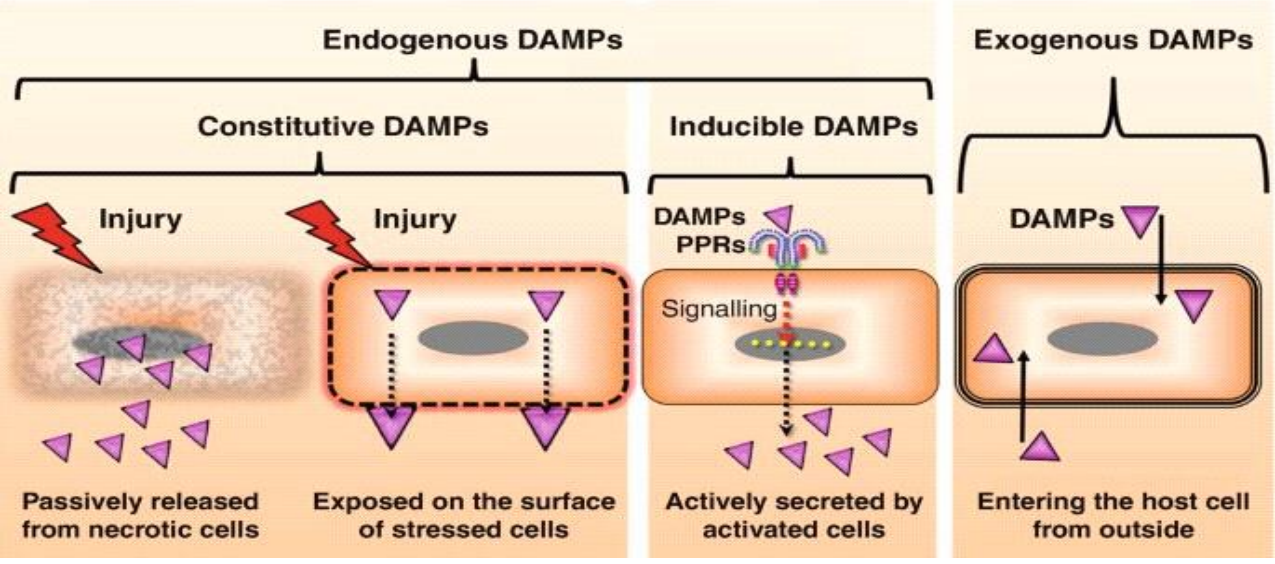

Fig (5) showing role of DAMPS and PAMPS in ACALF (60)

• ***Outcomes of systemic inflammation:***

1\Tissue hypo perfusion:

PAMPs and inflammatory mediators can induce nitric oxide **(NO)** synthase in splanchnic arteriolar walls. The resulting NO overproduction causes splanchnic vasodilation which decreases effective arterial blood volume, triggering homeostatic over activation of the endogenous neuro humoral vasoconstrictor system ,Neuro humoral mediators then cause intense vasoconstriction, particularly in the renal circulation, resulting in kidney hypo perfusion, decreased glomerular filtration rate and acute kidney injury **(AKI).(61)**

2\Immune-mediatedtissue damage:

Like sepsis in the general population, ACLF is commonly associated with blood leukocytosis, comprising activated immune cells that may migrate into tissues and cause immunopathology **(61)**

There is some evidence for this hypothesis in the context of cirrhosis. For example, tumor necrosis factorand Nuclear factor kappa-light-chain-enhancer of activated B cells **(NF-κB)** -dependent signaling pathways may play a role in impaired left ventricular contractility**(62)**

In NO-mediated pulmonary dysfunction and macrophage accumulation in lung microvasculature, and in hepatocyte apoptosis(63**)**.

Like sepsis- induced AKI, ACLF-associated AKI may not only involve tissue hypoperfusion but also capillary leukocyte infiltration, vascular micro thrombosis, and cell apoptosis**.(64)**

Moreover, the direct inflammatory damage to tissues and cells leads to the release of a huge amount of circulating cellular products, which act as DAMPs on immune cell receptors. Therefore, vicious cycle sustains and exacerbates inflammatory responses, providing the link between systemic inflammation, cell injury and organ failure**(55)**

2\Mitochondrial dysfunction

In ACLF, peripheral organs may have a marked decrease in mitochondrial fatty acid – oxidation in peripheral organs, resulting in decreased oxidative phosphorylation and ATP production. These findings suggest that defective energy production may play a role in the devel-opment of organs failure in ACLF**(65).**

3\Immunosuppression in ACLF

Patients with ACLF, 90-day mortality was higher in those who develop secondary infection than in those who remain free of this complication during the entire period of follow-up,indicating the extreme severity of secondary infection in this context. **(66)**

More importantly, the high risk of secondary infections indicates that patients with ACLF are immunosuppressed and supported by high level of immune suppressive molecules interleukin 10(**67).**

There are findings suggesting that, in ACLF, some subsets of immune cells have defective antimicrobial functions that contribute to the high risk of secondary infection such as Defective responses to **PAMPs** have been shown in macrophages derived from circulating monocytes obtained from patients with ACLF.

Finally, studies have shown that neutrophils in patients with decompensated cirrhosis have a marked defect in both the production of antimicrobial superoxide anion and bactericidal activity**.(68)**

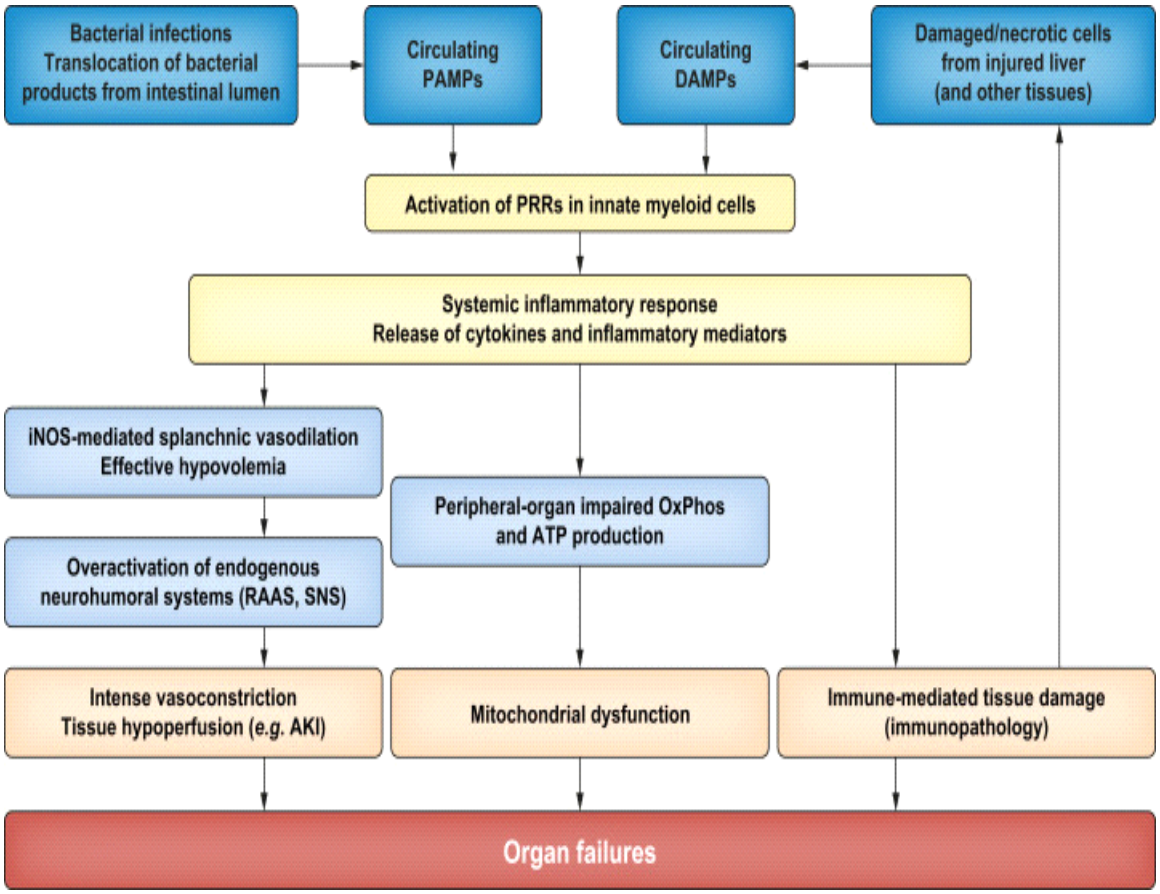

Fig (6) showing mechanism s of organ failure in ACALF (62)

- **COMPLICATIONS IN ACLF:**

1\Bleeding

Acute variceal bleeding is a serious complication of liver cirrhosisresulting from portal hypertension. Variceal bleeding is often associated with ACLF **(69).**

Patients with ACLF display an imbalance in systemic and hepatic hemodynamics with severe portal hypertension and worsening of systemic vasodilation**(70).**

The increased portal pressure developas a consequence of hepatic and systemic inflammation,reduced hepatic perfusion, and high intrahepatic resistance.

As patients with ACLF have high baseline hepatic venous pressure gradient and lower hepatic blood flow, the chances of variceal bleeding are also high **(71)**

Although a significant progress has been made in the treatment of acute variceal bleeding including trans jugular intrahepatic portosystemic shunt **(TIPS)**,endoscopic treatment, and drug therapy, 10–20% of the patients experience treatment failure that associate with a high short-term risk of further liver decompensation and death **(72)**

Recently, a study reported the prevalence of ACLF in patientswith acute variceal bleeding and found its association with rebleeding and mortality. ACLF nearly doubled the risk of re bleeding. Patients with ACLF with variceal bleeding may benefit from the placement of TIPS. In fact, the insertion of TIPS improves the 42-day and the 1-year survival in patients with ACLF Also, preemptive placement of TIPS is helpful in patients with ACLF with acute varicealrebleeding. **(72)**

2\Hepatic Encephalopathy

Hepatic encephalopathy (HE) is another frequent manifestationof ACLF. Patients with HE manifest a range of neuropsychiatric symptoms including sensory abnormalities, psychomotor dysfunction, and impaired memory**(73)**

Hyper ammonemia, systemic inflammation including sepsis, bacterial translocation, insulin resistance, and oxidative stress remain as key factors in the development of HE, as result of cerebral edema and inflammation **(74)**

During liver failure, reduced usage of ammonia as a substrate in the ammonia detoxification pathway (urea cycle) and portosystemic shunting increases ammonia accumulation in the systemic circulation **(75)**

Also, impaired hepatic metabolism leads to decreased elimination of nitrogen-based waste products such as ammonia which crosses the blood–brain barrier, where it combines with glutamate to form glutamine **(76)**

Cerebral accumulation of glutamine employs an osmotic effect that leads to increased retention of water in the brain, resulting in swelling and cytotoxic edema. HE in a hospitalized cirrhotic patient is related to high mortality that further increases in case of patients with ACLF **(74).**

Existence of severe HE in cirrhotic patients requires management in the ICU, and patients frequently require tracheal intubation for airway protection **(77).**

. For the precise management of HE, the early step is to identify and reverse any precipitating event such as infection or bleeding **(78).**

Therapies lowering ammonia are commonly used. Moreover, lactulose, a non-absorbable disaccharide that converts into short-chain fatty acids by the colonic microbiome generates an acidic environment, leading to the inactivation of ammonia-producing colonic bacteria, and the conversion of ammonia to non-absorbable ammonium **(79).**

Antibiotics are also recommended, generally in combination with lactulose that is helpful in reducing mortality and the length of hospital stay in comparison with lactulose alone **(80).**

As ammonia is considered a key participant in the pathogenesis of HE, antibiotics that reduce the ammonia-producing enteric bacteria including, neomycin,and metronidazole are used in combination with or without lactulose **(81).**

These antibiotics serve as second-line agents; not used for long-term use due to nephrotoxicity, ototoxicity, and neurotoxicity. For instance, neomycin is ototoxic and nephrotoxic, whereas metronidazole has neurotoxic effects. Another antibiotic Rifaximin, non absorbable oral antimicrobial agent, is highly efficacious in treating HE through eliminating ammonia-producing colonic bacteria, resulting in reduced ammonia concentration.

Rifaximin is poorly absorbed and has minimal systemic bioavailability which favors its long-term use than the other antibiotics ,now considered as 1 st line agent **(82).**

3/Concomitant Bacterial Infection:

Bacterial infections play an essential role in the development and further progression of ACLF, and participate either as a key precipitating event or as a complication **(83).**

At the time of ACLF diagnosis, approximately 37% of the patients exhibit bacterial infections, whereas 46% of the remaining patients with ACLF develop bacterial infections during the 4 weeks follow-up , Both Gram-positive and Gram-negative bacteria contribute to the infection **(84)**

The incidence of Gram-positive bacterial infections mainly Staphylococcus are increasing than the Gram-negative bacterial infections. The common Gram- positive bacteria includes Staphylococcus aureus and Enterococcus **(85)**

S.aureus causes respiratory tract and also skin infection, whereas Enterococcus frequently causes urinary tract infection. Besides, due to the improper use of antibiotics, antimicrobial resistance including methicillin-resistant S. aureus (MRSA) and Vancomycin-resistance Enterococcus (VRE) is also increasing in patients with cirrhosis **(86)**

The common Gram- negative bacteria causing infections include Escherichia coli and Klebsiellapneumoniae, E. coli leads to spontaneous bacterial peritonitis (SBP), whereas Klebsiellapneumoniae is a common cause of pneumonia. **(87)** .

Another Gram –negative bacteria Acinetobacter causes respiratory tract infection,urinary tract, and also skin infections, pneumonia, and SBP are predominant and complicate the condition of these patients**(88).**

In fact, the severity of ACLF measured by the prevalence of organ failure and mortality was higher in patients where ACLF was caused by an infection in comparison to those withnon-infectious etiologies**(89).**

Cirrhotic patients, especially the decompensated ones, are extremely susceptible to develop bacterial infection due to impaired gastrointestinal barriers and increased gut permeability that allows bacterial translocation to the surrounding tissues and end up in the blood stream leading to systemic inflammation, sepsis, and ACLF development **(90).**

Continuous translocation of bacteria and its products stimulate the immune cells after identification by pathogen-recognition receptors, typically **TLRs,** causing overwhelming inflammatory response via producing inflammatory cytokines **(91).**

High levels of circulatory pro inflammatory cytokines induce systemic inflammation and increase disease severity. Further, systemic inflammatory responses encourage organ damage through oxidative stress, endothelial dysfunction, and reduced organ perfusion. Also, pathogen and pathogen-derived endotoxins are efficient in promoting direct tissue damage **(92).**

In general, hepatocytes are moderately protected against LPS-induced tissue damage via the induction of **NF-κB pathway**; however, this mechanism is impaired in cirrhotic patients causing direct tissue damage **(93).**

Moreover, translocation of bacteria or their PAMPs impair the contractility of mesenteric vessels that supply blood to the small and large intestines and increase portal hypertension in cirrhotic patients, which further affect the microbiota and increase bacterial translocation(**94).**

Studies believe that liver, intestinal barrier and microbiota, and immune response preserve equilibrium through complex interactions, and perturbation in this balance leads to increased gut permeability, althoughthe precise mechanism is not clear.Earlydiagnosis and appropriate antibiotic use on time are critical factors to improve the prognosis of patients with bacterial infections. Also, biomarkers of infection may aid in the early diagnosis of infection. Acute-phase proteins including C-reactive protein **(CRP)** and procalcitonin (**PCT)** are early markers of infection that are frequently used to diagnose the infection **(92)**

A study described that a value of **CRP** >12.15 mg/L is a good indicator of bacterial infection in patients with ACLF **(95).**

Unfortunately, due to the increased use of antimicrobial agents, antimicrobial resistance has increased over the years. In fact, a study advocated against the use of antibiotics except in distinct conditions such as gastrointestinal bleeding, history of SBP, and ascites fluid protein concentration <1.5 g/dL, However, that cannot

be considered in patients with septic shock, as each hour delay in antibiotic treatment following identified hypotension can decrease patient's survival up to 7.6% for the first 6 h **(96).**

Due to the prolonged wait time in getting bacterial culture results, we lose vital time to treat the patient with antibiotics; however, antibiotic treatment without identifying the infection will put unwarranted stress on the liver; therefore, techniques that could identify the infections in short span of time are highly needed.

4/Fungal Infection:

Persistent impaired immune response and hepatocyte damage reduce the efficiency of inhibiting and clearing the pathogen in **ACLF**.

A study reported the occurrence of invasive fungal disease in 43% of patients with ACLF and observed higher mortality in these patients than those without the invasive fungal disease **(97).**

Candida as well as Aspergillus species are the common infections in **ACLF** and primarily infect urinaryand respiratory tracts,Like a bacterial infection, a fungal infection could act as the main precipitating event in **ACLF**; however, the mechanism is not well-recognized**. (98)**

It is believed that the exacerbation of **ACLF** induces immune paralysis, which can lead to invasive fungal infection. In addition, the invasive fungal disease is also responsible for increased inflammatory cytokine response that further augments organ failure. To identify the fungal infection, specific tests including fungal culture, serologies, and fungal tissue staining are required**(98)**

The invasive fungal infection is diagnosed by 1,3-β-D-glucan and galactomannan index. Recently, it has been shown that bacterial and fungal infections are associated with poor clinical course and high 28 and 90-day mortality **(99).**

Fungal infections not only increase short-term (28 days) and medium term (90 days) mortality, but also enhance the risk of 1-year mortality. The identification of an infection at the initial stage is the most challenging. The current approach is to culture the patient's sample, which is a time-consuming process and susceptibleto cross contamination. Therefore, it is critical to identify the infection based on the other clinical markers. Currently, the most common indicators of infection are systemic inflammatory response syndrome (**SIRS), PCT**, serum lactate **(100).**

- **Prognostic indices for risk stratification in patients with acute-on-chronic liver failure;**

Several scores have been developed and proposed to assess patient prognosis and help clinician decision-making.

European investigators have developed a score named CLIF-C ACALF score (Chronic Liver Failure- Consortium) that predicts mortality in patients with ACLF. This score was based on the CLIF-C OF score and enriched with the 2 best independent predictors of death in the CANONIC cohort: age and white blood cell-count, a marker of systemic inflammation . Both scores can be found and calculated on the EF-CLIF website (http://www.efclif.com). The accuracy for predicting death was better with the CLIF-C ACLF score than with other scores, including the MELD, MELD-Na, Child-Pugh and CLIF-C OF scores**. (51)**

The CLIF-C ACLF score has also been shown to be a better predictor of mortality than the usual ICU prognostic scores (including the SOFA and APACHE II scores). Moreover, the kinetics of the CLIF-C ACLF score during ICU stay reliably predicted patient outcome**(101).**

Investigators of the **COSSH** have developed the **COSSH-ACLF(Chinese Group on the Study of Severe Hepatitis B score**),based on a modified **CLIF-C OF** score, including specific risk factors for mortality observed in patients with **HBV-ACLF**.

The new score evaluated in patients with HBV-related liver disease, showed higher predictive value for 28-day and 90-day mortality compared to other scores, including **CLIF-C ACLF, CLIF-C OF, MELD, MELD-Na** and Child-Pugh scores. **(102).**

Investigators of the **AARC** developed and validated an **AARC ACLF score** (Asian Pacific Association for the Study of the Liver ACLF Research Consortium)**.(48).**

Baseline total bilirubin, HE West-Haven grade, INR, serum lactate and creatinine were found to be independent predictors of 28-day mortality in patients with ACLF according to AARC criteria.

The individual parameters were then scored from 1 to 3 considering their predictive accuracy for a low (<15%), medium (around 50%) and high (>80%) 28-day mortality rate. Therefore, the **AARC ACLF score** ranges from 5 to 15. Patients with **ACLF** were then categorised as grade 1 (5-7 points), grade 2 (8-10 points) or grade 3 (11-15 points) (with a 28-day mortality rate of 13%, 45% and 86%, respectively .

In patients with **ACLF** according to **AARC criteria**, the score was found to be superior to **MELD** and **CLIF-SOFA** score in predicting short-term mortality. The score can be found and calculated on the **APASL-AARC**

- **Therapeutic options for ACALF :**

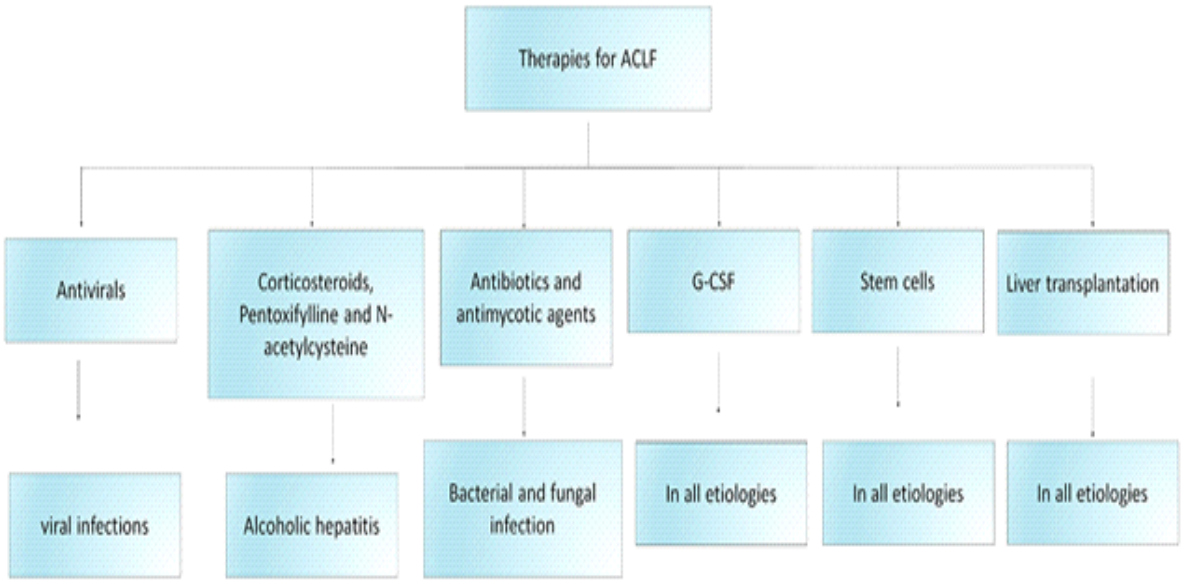

Fig (7) Different lines of treatment of ACALF according to etiology (103)

No specific treatment for patients with ACLF, most important item is the early detection of the syndrome, the treatment of precipitating factors (bacterial infections, treatment for HBV, corticosteroids in alcoholic hepatitis, etc.) and support treatment for the various types of organ failure.

Patients with ACLF, especially those with ACLF-2 and ACLF-3, should be managed in intensive or intermediary care units and, unless there are complications, should be transferred to hospitals with an LT facilities .

1.Treating acute precipitants, ***Antimicrobial therapy***

In a recent study, about 37% of patients with ACLF presented with a bacterial infection at diagnosis. Furthermore, 46% of the remaining patients with ACLF developed bacterial infections within the next 4 weeks.(**84)**

Multidrug-resistant (MDR) pathogens are involved in one-third of cases with differences in prevalence according to region. **(104)**

A systematic search for infection, including microbiological and cytological examination of ascetic fluid, should therefore be systematically performed at admission. An empirical antibiotic therapy tailored to the suspected site of infection and the local ecology should be rapidly initiated.

Broad-spectrum molecules should be preferred in case of severe infection or in the presence of risk factors for **MDR** pathogens.

2.Organ support

A. Intravenous fluids

Fluid therapy should use crystalloids, while balanced salt solutions may limit the risk of hyperchloremic acidosis and subsequent adverse kidney events

Beneficial effects of albumin resuscitation have been demonstrated in patients with cirrhosis and may be related to more than volume expansion, besides the overall decrease of albumin, the function of albumin is also impaired with alterations in its chemical structure, resulting in reduced binding capacity to bacterial products, reactive oxygen species, and other mediators involved in ACLF. **(105).**

Some studies suggested that albumin may modulate systemic oxidative stress and inflammation or restore immune defense. **(106).**

Current guidelines recommend the infusion of human albumin in 3 clinical situations:**(107):**

1. After high-volume paracentesis (more than 4-5 litres, 8 g of albumin per litre of ascites removed);
2. In patients with **AKI** stage 2-3 of the modified KDIGO classification specifically redefined by the International Club of Ascites (1 g/kg/day for 2 days), and inpatients with hepatorenal syndrome (1 g/kg on day 1 and then 20-40 g/day), associated with vasoconstrictors (terlipressin 2 mg/24 h as first choice);
3. In patients with **SBP** (at a dose of 1.5 g/kg at diagnosis and 1 g/kg on day 3).

In patients with cirrhosis and infections unrelated to SBP, albumin treatment does not improve survival but is associated with lower systemic inflammation, a higher rate of ACLF resolution and a lower rate of nosocomial infections. **(86).**

B. Renal replacement therapy

Term used to refer to modalities of treatment that are used to replace the waste filtering functions ofnormal kidney (haemodialysis,perotineal dialysis, hemofiltration ,renal transplantation)

c.Extracorporeal liver support:

The Best-known devices are based on the principle of albumin dialysis. Two multicentre randomised European trials in patients with acutely decompensated cirrhosis compared these systems with standard medical treatment. These studies showed an improvement of cholestasis and hepatic encephalopathy in patients treated with albumin dialysis but did not demonstrate any benefit on 28- and 90-day survival**. (108)**

More recently, the use of an artificial liver support system was associated with improved short-term survival (14- and 28-day) in patients with ACLF and multiple organ failures in a retrospective study 68 and a meta-analysis, therefore, these devices may be interesting as a bridge to liver transplantation or recovery**. (109)**

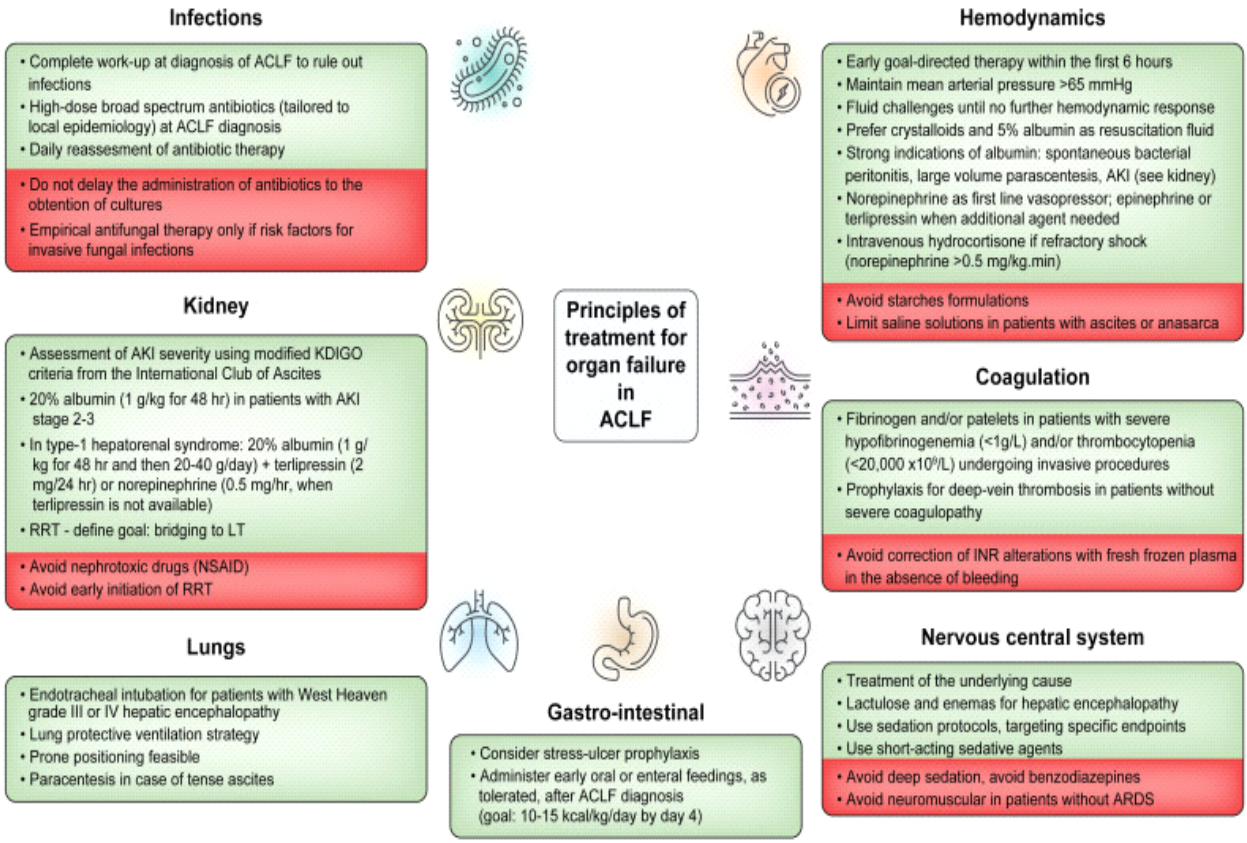

Fig (8) supporting treatment for organ failure (110)

3.Livertransplan

An LT is the optimal and definitive treatment in patientswith ACLF.patients with ACLF should be evaluated for an LT. However, transplantation in patients with ACLF is complex due to narrow time and multiple organ failure.

Furthermore, the waiting list mortality rate for these patients is very high so, opinions have emerged suggesting that to improve the prognosis for ACLF, especially in serious stages, those on the LT waiting list should be having priority. **(111)**

The results are improved regarding patients with ACLF-2 or ACLF-3, the 28-day survival without an LT was less than 20%,but increased to 80% in patients who received a transplant, the median time between ACLF diagnosis and transplantation was 11 days (1---28 days). **(52)**

However, it is not feasible in all patients because of its high cost and lack of liver donors. Since, liver transplantation may be a lifesaving treatment, critical evaluation of final indication of liver transplant such as high MELD score, complications due to cirrhosis such including ascites, varicealhemorrhage, and HE should be considered **(112).**

Patients with high MELD score have quick access to liver transplant; though, the requirement of suitable organ donors impose a major limitation. Patients with ACLF with grade 1 and 2 display a similar post transplant survival rate as those without ACLF **(69).**

4. Stem-Cell-Based Therapies:

A.MSCs(mesenchymal stem cell therapy)

Have massiveexpansion potential in the culture system and play a crucial role in tissue repair and regeneration by differentiating into several cell types and replacing the injured tissues **(113)**

The homing potential of MSCs to the site of injury extended the spectrum of therapeutic application, after homing into the liver,MSCs trans differentiate into hepatocytes in the local microenvironment and improve hepatocyte damage and promote liver regeneration. There are ongoing phase II clinical trials (NCT04229901, NCT02946554) investigating the efficacy of HepaStem cells, a highly advanced cell therapy platform comprising human-liver- derived MSCs obtained from healthy donors and expanded in the lab.**(114)**

After intravenous administration, HepaStem cells migrate to the liver through circulation where they perform various functions including the downregulation of pro inflammatory response, inhibition of hepatic stellate cell (HSC) activation, and reduction of collagen secretion, ultimately reducing fibrosis. **(115)**

MSCs secrete several growth factors which stimulate resident cells and induce matrix remodeling to promote the differentiation of native progenitor cells and initiate the recovery of injured cells ,In addition, they also possess antioxidant properties and cytoprotective effects by inducing antioxidant response **(116).**

Umbilical-cord-derived **MSCs** and also allogenic ABCB5-positive MSCs that improve liver fibrosis enhance regeneration, suppress inflammation, and downregulate Notch and Stat1/Stat3 signaling in rats are under clinical trials (NCT04822922, NCT03860155) for the treatment of patients with ACLF. **(117)**

Although the mechanisms of **MSCs** have been well-described in CLDs, the mechanistic approach of **MSCs** in the treatment of ACLF is not well-documented since it was recently introduced as a therapeutic intervention for **ACLF** and clinical trials are ongoing.

It is believed that immunomodulatory and anti-inflammatory function of **MSCs** relieve hepatic inflammation, improve liver function, decrease the incidence of infection, and enhance survival rate as shown in a prospective randomized controlled clinical trial which investigated the safety, efficacy, and outcome of **MSCs** in **HBV**-related **ACLF** after intravenous infusion **(118)**

The study reported that there were no infusion-related side effects except more frequent fever than patients who received standard medical therapy. Clinical laboratory measurements including total bilirubin and model for end-stage liver disease scores were improved and the incidence of severe infections was decreased (**(118)**

In fact, multiorgan failure and severe infection-related mortality were significantly lower in the MSC group. Importantly, the 24-weeks survival rate of the **MSC** group was higher (73.2%) than the standard medical treatment patients' group (55.6%). Another study also examined the long-term efficacy of autologous bone marrow mononuclear cells (**BM-MNCs**) transplantation through the hepatic artery and checked the improvement in terms of hepatic functions and decreasing complications in patients with decompensated cirrhosis**(119)**

The study reported that the efficacy of **BM-MNCs** transplantation persisted for 3–12 months in comparisonwith the control group. Serious complications including HE and SBP were declined significantly; however, these improvements vanished after 24 months of transplantation.

B.Granulocyte-Colony Stimulating Factor Therapy:

Granulocyte colony stimulating factor (G-CSF) is a glycoprotein, which stimulates the bone marrow production of stem cells and granulocytes and releases them into the circulation ,One week of **G-CSF** treatment

increased leukocyte as well as neutrophil count and reduced disease severity indices in patients with **ACLF** ,**G-CSF** therapy also prevented the development of sepsis, **HRS** and **HE**, and improved survival of these patients **(120).**

CD34 expressing cells are generally considered to be hematopoietic stem cells that differentiate into all hepatic cell types and recover hepatic damage by inducing liver regeneration. We observed an increase in the mobilization of CD34+ stem cells after **G- CSF** treatment.

Moreover, **G-CSF** has immunomodulatory effects shown by an increase in myeloid dendritic cells and a decrease in **IFN-γ** producing T cells after **G-CSF** therapy in patients with ACLF, which is beneficial in terms of reducing **IFN-γ** mediated inflammation and hepatic damage in these patients **(121).**

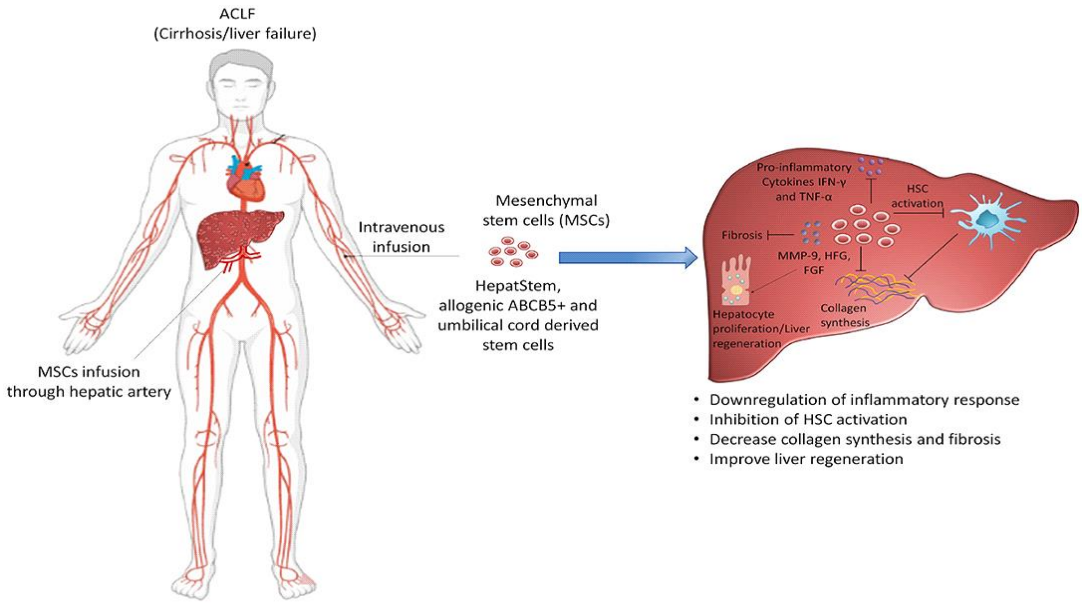

Fig (9) Role of MSCs in treatment of ACALF (113)

On the contrary, a recent European multicenter clinical trial reveals that **G-CSF** does not have any superior benefits than the standard medical treatment. The findings revealed that **G-CSF** is ineffective in improving patient survival and other clinical endpoints including **MELD score**, **CLIF-C** organ failure score, and the occurrence of infection, recommending **G-SCF** not be used as a standard treatment for **ACLF(122).**

To summarise, more studies are required that include a homogeneous population of patients with **ACLF** linked to different aetiologies in order to establish whether liver support systems can really play a role in managing these patients.

- **Prognosis**

ACLF is characterised by a high short-term mortality rate that ranges between 30% and 50%. As expected, patients with ACLF-3 present with the worst prognosis, compared to patients with ACLF-1 and ACLF-2 .**(123).**

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