Predictors of steroid non-response and new approaches in severe alcoholic hepatitis

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Severe alcoholic hepatitis (SAH) remains a disease with high mortality. Steroid is the mainstay and has been shown to give modest 28-day survival benefit in carefully selected patients, but no 90-day survival benefit. Since non-responders have high incidence of infections and increased mortality, it would be worthwhile to identify them before starting steroid therapy. A high and rising bilirubin, urinary acetyl carnitine >2,500 ng/mL, high asialoglycoprotein positive microparticles, and specific features in liver biopsy could predict steroid non-response at baseline. There is an ever-growing need to find new and effective therapies for SAH patients. Besides aggressive nutrition, granulocyte colony stimulating factor, fecal microbiota transplantation, and plasma exchange appear promising therapies and provide a hope for steroid ineligible or steroid non-responsive patients. Suppression of hepatic inflammation, preventing new bacterial or fungal infections, and enhancing liver regeneration will remain the key targets for next generation therapies. (Clin Mol Hepatol 2020;26:639-651)

Keywords: Hepatitis, Alcoholic; Steroids; Granulocyte colony-stimulating factor; Microbiota; Microvesicles

INTRODUCTION
The burden of alcohol-related liver diseases (ALD) is rapidly growing and is likely to increase in the coming years. With the availability of better treatment options for managing hepatitis B and C, alcoholic hepatitis (AH) related hospital admissions are adding substantial load on healthcare costs and utilization.¹,² The spectrum of ALD varies from fatty liver, steatohepatitis, severe AH, alcoholic fibrosis, compensated/decompensated cirrhosis, to hepatocellular carcinoma. ALD now contribute to 47.9% of all liver cirrhosis-related deaths globally.³,⁴ AH is the most florid manifestation of ALD with substantial morbidity, mortality, and financial burden.

AH is an acute form of alcohol-induced liver injury, ranges from mild to severe, and usually presents on the background of chronic liver disease. It is clinically defined as recent onset or worsening of jaundice in a patient with chronic heavy alcohol use until at least 6 weeks prior to presentation, elevated liver enzymes with

Abbreviations:
AH, alcoholic hepatitis; ALD, alcohol-associated liver diseases; BAG1, BCL2-associated athanogene1; BD, ballooning degeneration; CI, confidence interval; DCs, dendritic cells; FA, fatty acid; FGF, fibroblast growth factor; FMT, fecal microbiota transplantation; G-CSF, granulocyte colony stimulating factor; GM, gut microbiome; HMA, human mercaptalbumin; HNA, human non-mercaptalbumin; IL, interleukin; LCFA, long chain-fatty acid; MAS, macrophage activation syndrome; MD, Mallory-Denk bodies; MDF, Maddrey’s discriminant function; MELD, model for end-stage liver disease; MV, microvesicle; NAC, N-acetylcysteine; RCT, randomized control trial; ROS, reactive oxygen species; SAH, severe alcoholic hepatitis; SD, standard deviation; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment

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Editor: Byoung Kuk Jang, Keimyung University School of Medicine, Korea
Received: Jul. 30, 2020 / Revised: Aug. 14, 2020 / Accepted: Aug. 21, 2020

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an aspartate aminotransferase to alanine aminotransferase ratio of >1.5:1, with absolute values of these enzymes not exceeding 500 IU/L and exclusion of other liver diseases. Severe AH (SAH) is often a progressive disease with a 28-day mortality of 20–50%, hence a definite diagnosis is helpful. For a definite diagnosis of AH, a histological evidence of the disease is required besides clinical and biochemical criteria, as 10–20% of AH patients may have other liver diseases.

Predominant histological lesions that characterize AH include the coexistence of steatosis, hepatocyte ballooning, and inflammatory infiltrates, mainly with neutrophils, called satellitosis. Liver biopsy also helps in providing clues to the natural history and outcome of the disease. Presence of megamitochondria, polymorphonuclear leucocytic infiltration, and absence of fibrosis is associated with good prognosis, whereas presence of ballooning hepatocytes and bilirubinostasis is suggestive of poor prognosis. However, majority of the SAH patients have coagulopathy or ascites which are contraindications for percutaneous liver biopsy and necessitate transjugular route for obtaining liver tissue. However, due to invasive nature of the procedure and associated costs, often, the patients are started on treatment on the basis of ‘probable’ diagnosis of AH. This can pose challenges in some situations, especially in patients with obesity, diabetes, and associated drug-induced liver injury.

**ASSESSMENT OF SEVERITY OF AH**

An important part of therapy for any disease requires assessment of the severity of the disease and the appropriate time for initiation of the therapy. Assessing severity of AH can be done by using a combination of clinical and biochemical parameters. Maddrey’s discriminant function (MDF) is one of the first bedside tool, which is still used across the world, and a score of >32 categorizes the disease as SAH. The MDF however, has not been validated for assessing the treatment response. Model for end-stage liver disease (MELD) score, commonly used for organ allocation for transplant, has also been used. A MELD score of >18 indicates SAH and mandates specific therapy. Alternates like Glasgow alcoholic hepatitis and sequential organ failure assessment (SOFA) scores are dependent on baseline clinical and biochemical features.

**THERAPEUTIC OPTIONS FOR SEVERE AH**

In mild or moderate AH, abstinence does help to some extent. For severe AH, specific and effective therapy is required to suppress the ongoing inflammation and hepatic necrosis. Unfortunately, there are very limited options. Corticosteroids currently remain the first and probably the only accepted medical therapy for SAH patients.

**CORTICOSTEROIDS FOR AH**

Since AH is considered as a severe form of inflammatory disease of the liver, prednisolone with its anti-inflammatory action, has been used for over 40 years for such patients. Prednisolone however, provides only a modest short-term benefit. Lille score is used to assess the efficacy of steroid therapy and is calculated (based on age, baseline creatinine, albumin, prothrombin time, total bilirubin and repeat total bilirubin) at day 7. Those with Lille score >0.45 indicate poor response to corticosteroids and under 25% survival rate at 6 months. In a meta-analysis to predict treatment response to steroids, patients were classified as complete responders (Lille score <0.16), partial responders (Lille score 0.16–0.56), and null-responders (Lille score >0.56). Significant differences in survival (91% vs. 79% vs. 53%, P<0.0001, respectively) were observed. It is necessary to remember that steroids should be continued only in complete responders.

In a recent study of Shasthry et al. have reported that of the 430 hospitalized patients with SAH, only 132 (26.8%) were eligible to receive steroids. Of these, 99 patients (75%) responded to steroids; making a total of 331 of 430 (77%) as steroid non-responders. It has been shown that only 1–2% of severe AH patients actually undergo the definitive therapy, the liver transplant. This draws attention to the fact that nearly four out of five patients with SAH are deprived of an effective therapy.

Furthermore, infections are not uncommon after initiation of steroid therapy. Steroid non-response is associated with a high risk of infection and poor outcomes. In a prospective study, around 25% of patients receiving steroids developed infections. Median MELD and MDF were higher in these patients compared to those who were non-infected on steroids, suggesting that sicker group of patients are not suitable for steroids.

There is an unmet need to treat these large cohort of patients with severe AH with newer therapeutic strategies. Furthermore, even for the steroid-eligible patients, it would be highly desirable, if predictors of non-response to steroids could be defined prior to initiation of steroid therapy.
IDENTIFICATION OF STEROID NON-RESPONDERS PRIOR TO THERAPY

If steroid non-responders can be identified prior to starting steroids, several adverse effects like development of new infections, gastrointestinal hemorrhage, hyperglycemia, etc. could be prevented.

Biochemical predictors

Persistently high and rising bilirubin is very suggestive of an unremitting inflammation, likely to be unresponsive to steroids.

Day 4 Lille score

Whether assessment of the same parameters which form the Lille score can reliably predict non-response at day 4 rather than day 7, was evaluated. The data showed that Lille score on day 4 was as good as day 7 to predict 3-month mortality and reduces unnecessary steroid exposure. However, these results have not been validated by other workers. In our own large experience (unpublished data), day 4 is not a very reliable indicator of response to steroids, and legitimacy to continue therapy for 7 days.

Baseline liver biopsy

A pre-therapy liver biopsy helps in stratification of SAH patients not only to assess the severity of liver disease, but also to predict response to therapy. Liver biopsy showing ballooning hepatocytes and bilirubinostasis, indicates a poor response to steroids. In one study, non-responders had higher ballooning degeneration (BD) (mean, 3.87 [standard deviation (SD), -0.91] vs. 2.92 [SD, -1.33], P =0.013) with increased density of Mallory-Denk bodies (MD) (mean, 2.27 [SD, -0.79] vs. 1.69 [SD, -0.97], P =0.028). A score derived using BD and MD (range, 0–8) had high sensitivity and specificity (81.2% and 64%, respectively), and negative predictive value (91.4%) to identify non-responders to steroids at baseline if the score is >5.

Metabolic markers for early detection of steroid response

Patients with SAH have a unique metabolic phenotype, one with severe inflammatory milieu and an impaired immune status. Whether baseline metabolic phenotype could identify non-responders and those with unfavorable outcome was investigated. Baseline urine metabolome of SAH patients was subjected to ultra-high performance liquid chromatography and high-resolution mass spectrometry. Nine urinary metabolites linked to mitochondrial functions significantly discriminated non-responders, most importantly the increased acetyl-L-carnitine (12-fold) levels. A baseline urinary acetyl-L-carnitine level predicted non-response with a receiver operating characteristic of 0.96 (95% confidence interval [CI], 0.85–0.99) and a hazard ratio of 3.5 (95% CI, 1.5–8.3) for the prediction of mortality in patients with SAH. Acetyl-L-carnitine levels of >2,500 ng/mL reliably segregated survivors from non-survivors (P<0.01, log-rank test) in the study cohort.

This low cost test, if validated in other studies, could be of help in selection of patients for steroid therapy.

Specific microvesicle signatures

Microvesicles (MVs) reflect the cellular stress and the disease conditions. In one study, pre-therapy peripheral plasma MV levels of haematopoietic stem-cells (CD45+ CD34+; 116.8 vs. 13.4 MV/µL; P =0.0001) and hepatocytes (ASGPR+; 470 vs. 361 MV/µL; P =0.01) were higher in steroid non-responders, compared to responders (Fig. 1). In fact, the two together could predict steroid non-response in 94% patients at baseline in peripheral plasma. The basis for these observations lies in the fact that the MVs from SAH patients, trigger more (P =0.04) reactive oxygen species (ROS) generation, tumor necrosis factor-α production (P =0.04) and up-regulate pro-inflammatory cytokine related genes in neutrophils in vitro. The source of these MVs was also validated with higher levels in hepatic vein plasma of non-responders. The MVs from hematopoietic cells suggests that the bone marrow cells do migrate to liver, but probably, a fair proportion is unable to induce effective regeneration due to an unfavorable milieu and, hence possibly perish as assessed by high MV levels in hepatic vein in non-responders. More such biomarkers are needed for proper patient selection for steroid therapy.

Baseline hepatic gene expression

Whether variation to steroid response in SAH patients has a genetic basis, is largely unknown. This was explored by investigating the hepatic transcriptome in patients originating in different countries. Though at presentation the patients were similar, the responders and non-responders had distinct patterns of gene expression. There were >1,100 genes that were overexpressed (>2-fold).
fold, \( P < 0.05 \) in non-responders in comparison to responders. Importantly, the glucocorticoid receptor (NR3C1) expressed a significantly reduced amount of the alpha isoform in non-responders. Additionally, in one population the BCL2 associated athanogene-1 (BAG1) was significantly increased in non-responders. BAG1 is known to cleave the glucocorticosteroid receptor (Fig. 2). This data shows BAG1 as a promising candidate to be explored in a larger population to segregate steroid non-responders at baseline.

**Oxidized albumin forms**

Circulatory albumin is a potent ROS scavenger. However, structural modifications in albumin may modulate its antioxidant and immune-regulatory properties. Such alterations in circulating albumin in SAH patients along with their contribution to neutrophil activation, intracellular stress, and alteration in associated molecular pathways have been assessed. The 3 isoforms of circulating albumin - human mercaptalbumin (HMA) and human non-mercaptalbumin (HNA1 and 2) were measured in SAH patients. Albumin isoforms showed that total HNA1 was significantly \( P < 0.05 \) higher in SAH (44.5%) patients compared to healthy controls (28.6%). Additional modifications like glycosylated HMA was also higher in SAH than controls (17.4% vs. 10.6%, \( P < 0.05 \), respectively).

**NEW APPROACHES TO TREATMENT OF AH**

Conceptually, the approach to manage SAH includes attempts to abrogate the severe inflammation and necrosis due to alcohol-induced injury, removal of the toxic metabolites produced due to severe inflammation in AH, stimulate liver regeneration despite a toxic milieu of a failing liver using endogenous or exogenous growth factors and modulation of gut bacteria to availability of appropriate nutrition and immune modulation. (Fig. 3). Unfortunately, at present, there are limited therapeutic options for patients, besides steroids. Moreover, for those who do not respond to steroids, the choices are meager. In the past decade, some progress has been made in this direction (Fig. 4).

**Granulocyte colony stimulating factor (G-CSF)**

G-CSF (also known as CSF3) is a colony stimulating factor which stimulates bone-marrow precursor cells to produce granulocytes and stem cells, and release them into the blood. Inflammation and impaired liver repair and regeneration are the major factors responsible for liver failure in patients with AH. A state of hyper-inflammatory response leading to progressive hepatocyte damage is accompanied by skewed and deficient immune system in SAH patients. Agents that can ameliorate gut-derived infection and inflammation, and enhance native liver

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**Figure 1.** ASGPR-positive hepatocyte MPs are increased. ASGPR, asialoglycoprotein; MPs, microparticles; NR, non-responder; HC, healthy control.
regeneration including bone marrow response are needed. The maximal level of CD34+ cells are released on day 5 after daily G-CSF administration, and the levels fall rapidly on subsequent days despite a continued rise in white blood cell counts.24

G-CSF regulates innate immune cell response by modulating the production, maturation, migration, and function of neutrophils, monocytes and dendritic cells (DCs). Excessive alcohol consumption impairs this granulopoiesis and the functionality of granulocytes resulting in defects in immune defense and susceptibility to serious infections.25 Despite, peripheral neutrophilia, AH patients are susceptible to infections, due to neutrophil dysfunction in the form of neutrophil resting oxidative burst greater than or equal to 55% and reduced phagocytic capacity of <42% due to persistent endotoxemia.26 This is partly due to the defects in the interleukin (IL)-33/suppression of tumorigenicity-2 pathway which is involved in sepsis control and is associated with decreased C-X-C motif chemokine receptor 2 expression on the cell surface and lower neutrophil migration capacity.27 It is therefore important to identify patients in the golden window,28 the time between the inflammatory response and the onset of sepsis, as the ideal time for therapeutic interventions for SAH. It is probably the most suitable time to introduce the G-CSF therapy, to prevent infections and enhance liver regeneration.

Locally produced G-CSF within the injured tissue also affects the function of recruited neutrophils and DCs. The frequencies of intrahepatic myeloid DCs and plasmacytoid DCs are lower in non-survivors compared to survivors. Intravenously given G-CSF has been shown to enhance the recruitment of plasmacytoid DCs, nearly 12-folds, resulting in reduction in the intrahepatic gamma interferon production and amelioration of hepatic injury (Fig. 5).29 G-CSF mobilizes bone marrow hematopoietic stem cells into the circulation by reduced C-X-C motif chemokine ligand 12 levels and adhesive action and facilitates their release into the circulation. G-CSF also stimulates the peripheral sympathetic nervous system, increasing catecholamine concentrations.30 G-CSF facilitates liver regeneration by migration of bone marrow-derived progenitors to the liver and by enhancing hepatic progenitor cell proliferation by its chemoattractant and mitogenic effects.31

Clinical trials exploring G-CSF therapy in AH

In a randomized trial of patients with acute-on-chronic liver failure, mostly due to alcohol, G-CSF was able to reduce the incidence of new infections, hepatorenal syndrome and hepatic encephalopathy with an improved 28-day survival compared to placebo (69% vs. 29%, P<0.001).32 Also, CD34+ hematopoietic cells were increased in liver biopsies with post G-CSF therapy. The drug was given as 5 mg/kg/day subcutaneously for 5 days and then once on alternate day for a period of 28 days (total 12 doses). It was well tolerated and no major side effects were reported.

G-CSF (given as 5 mg/kg, twice daily for 5 days) in another study on 46 SAH patients with a mean MDF of 85, was found to

Figure 2. Conformationally folded GR binds to glucocorticoid (i) and shifts to nucleus, where the receptor dimerizes and binds to co-factors (e.g., NFκB) to inhibit pro-inflammatory gene transcription. BAG1 cleaves GR (ii) in cytoplasm and prevents its downstream suppression leaving pro-inflammatory gene transcription active. GR, glucocorticoid receptor; BAG1, BCL2-associated athanogene 1.
improve CD34+ cells in peripheral blood at the end of therapy along with improved 90-day survival (78.3% vs. 30.4%, P<0.001).33 These reports are mainly from Asian countries. In one study34 on 58 decompensated alcohol-related cirrhotics, isolated bone marrow derived mononuclear cells including CD34+ hematopoietic cell infusion in the hepatic artery did not show survival benefit or better regeneration on repeat liver biopsy. Similarly, when given in compensated cirrhotics, G-CSF therapy for 5 days followed by leukapheresis and intravenous infusion of CD133-positive hematopoietic stem cells on day 5, 30, and 60 did not show benefit. An important aspect to be noted is that the studies from Europe, had included entirely different set of patients, not those with active AH, and with many having had sepsis. A recent meta-analysis has highlighted the differences in various studies.35 Based on multiple randomized control trials (RCTs), it can be concluded that in carefully selected patients of AH, G-CSF therapy can help in reducing

Figure 3. Rationale approaches for treating severe alcoholic hepatitis. SAH, severe alcoholic hepatitis; DAMPs, damage-associated molecular patterns; FMT, fecal microbiota transplantation; GCSF, granulocyte colony stimulating factor; FPSA, fractional plasma separation and adsorption.

Figure 4. Proposed algorithm for treatment of patients with severe alcoholic hepatitis; steroid non-responders or ineligible patients could be counseled to try experimental therapies. DF, discriminant factor; GCSF, granulocyte colony stimulating factor. *A baseline assessment of likely steroid non-response could be performed.
inflammation, incidence of infection and sepsis, reducing liver severity scores and possibly improve the survival (Fig. 6).

For steroid non-responders, there are few choices. In the only available study, GCSF was evaluated in steroid non-responsive SAH patients. Shasthry et al. in a group of 32 steroid non-responders, showed that GCSF administration reduced the MDF and MELD scores and in-tum the mortality, compared to the placebo (35.7% vs. 71.4%, P=0.04).

**Patient selection for G-CSF therapy**

Patients with severe AH, not amenable to steroids treatment or steroid non-responders can be considered for G-CSF administration. However, as noted in most of the RCTs, patients with MDF >90, hemoglobin <8 g/dL, high ferritin levels, macrophage activation syndrome (MAS), evidence of iron overload, splenomegaly, ascites, total leucocyte count of >40,000/mm³, infections, culture positive sepsis, acute kidney injury, hemodynamic instability, human immunodeficiency virus seropositivity, high adenomatous colorectal polyps or suspicion of malignancy, should be excluded from G-CSF therapy. In fact, it is safer and the response is more likely, if the MELD is <15. A good bone marrow is very helpful in stimulating the hepatic regeneration. Patients with severe sarcopenia, osteoporosis and anemia are less likely to have a responsive bone marrow. Also, a careful screening for the presence of infections should be undertaken. The efficacy of G-CSF is mainly in prevention of infection, rather than in the management of sepsis in SAH patients, where systemic inflammatory response syndrome (SIRS) can get exaggerated.

**Modulation of gut microbiota**

The gut microbiome (GM) plays a major role in liver disease. A chronically altered and unhealthy microbiome contributes to the development and perpetuation of liver disease, and this is probably true for SAH. The GM digests the ingested alcohol, with an increase in acetaldehyde concentrations in the gut lumen, and dis-

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**Figure 5.** G-CSF increases DC population which reduces IFN-γ resulting in improved survival. Adapted from Khanam et al. G-CSF, granulocyte colony stimulating factor; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; IFN-γ, interferon gamma.

**Figure 6.** G-CSF has multifaceted applicability in severe alcoholic hepatitis patients; reduction of inflammation, prevention of infection and hepatic regeneration. Adapted from Sarin and Choudhury. G-CSF, granulocyte colony stimulating factor; HSC, hematopoietic stem cells; MAPC, multipotent adult progenitor cells; GF, growth factor; HPC, hepatic progenitor cells; BMDC, bone marrow derived cells.
ruption of the mucosal barrier, permitting translocation of viable bacteria and their products, into portal circulation. Locally in the gut, the overgrowth of pathogenic species changes the bile acid composition, enteral metabolites, and entero-hepatic circulation. Resultant immune dysregulation makes the host susceptible to infections and continued hepatic injury. Patients with AH, have a pathobiont of their own. This alcohol pathobiont represents altered bacteria, their metabolites, and imbalanced cytokine milieu. Transferring intestinal microbiota from a patient with severe AH into mice, can lead to the development of severe liver inflammation in the animal.

Quantitative and qualitative variations in fecal lipid metabolites like the short-chain fatty acids butyrate and propionate have been reported in rats following chronic ethanol administration. Lactobacillus species use saturated long chain-fatty acids (LCFAs) as a source of energy, which are consequently reduced, resulting in tight junction barrier disruption. In mice models, saturated LCFA supplementation has been shown to restore eubiosis, stabilize the intestinal gut barrier, and reduce ethanol-mediated liver disease (Fig. 7). Ethanol exerts a direct effect on the saturated fatty acid (FA) biosynthetic gene in intestinal bacteria, independently of the host. Hepatocyte synthesized bile acids are secreted via bile duct into the duodenum facilitating digestion, and absorption of lipids, and lipid soluble vitamins. The bile acids modulate the gut microbiota through direct and indirect mechanisms. The gut microbiota then metabolizes primary bile acids into secondary bile acids. More than 95% bile acids actively taken up by enterocytes in the terminal ileum are exported through the basolateral membrane into the portal vein. Bile acids bind to the farnesoid X nuclear receptor and induce its target gene, the fibroblast growth factor (FGF)-19 in humans (or in mice, FGF-15). Both, FGF19/15 are receptor and induce its target gene, the fibroblast growth factor receptor and exerting beneficial effects on hepatic lipid metabolism.

AH-related dysbiosis is associated with an increase in Bilobed bacteria, Streptococci, Enterobacteria with concomitant decrease in anti-inflammatory Clostridium leptum or Faecalibacterium prausnitzii. The population density of these protective strains inversely correlates with clinical parameters such as bilirubin. Fecal microbiota transplantation (FMT) studies showed that there is a distinct microbiota signature associated with severe AH, and that a specific alcohol ‘pathobiont’ possibly exists. Identification of these microbial species would identify new targets and therapies.

Fecal microbiota transplantation

Alteration in the GM of a patient can be achieved by using probiotics, FMT or using genetically altered bacteria. While the later has not reached the stage of clinical application, the first two approaches have been evaluated in small sets of patients.

Probiotics have been shown in a small randomized trial to be of benefit in cirrhotic patients, including those with hepatic encephalopathy. No large clinical studies are available of the use of probiotics in SAH patients.

FMT has been used in multiple clinical conditions in gastroenterology. Its use was evaluated in a pilot study of eight steroid-ineligible SAH patients, and was compared to anecdotal controls. FMT showed significant survival benefit at day 90 with the donor microbiota co-existing in the recipients till 12 months.

It is known that subjects in the same household, living and eating the same food, generally share common bacterial taxa. We therefore preferred using FMT material from a related healthy individual living in the same household, with a presumption that the bacteria could adapt readily in the patient.

Recently, concerns have been raised about the safety of FMT therapy as fecal instillate may serve as a medium to carry pathogens. Infection with pathogenic Escherichia coli and development of adverse events in immunocompromised and immunocompetent patients indicates need for additional caution. However, risk of bacterial and fungal infections is high after steroid therapy in SAH patients, and may add to mortality. We have completed a large study on 112 SAH patients, comparing the safety and efficacy in 90 day-survival in those receiving steroids with healthy donor FMT. We serially assessed the recipients for the improvement in clinical and biochemical parameters.
changes in their GM, occurrence of adverse events, new complications, and infections. Since Lille score has been validated only for steroid therapy, new scores for assessing the therapeutic response to emerging therapies have also been developed. The initial results of the FMT in SAH patients are quite encouraging. Attempts have been made to inhibit the effects of pro-inflammatory IL-1 by using antagonist to its receptor, anakinra. In a clinical trial comparing anakinra efficacy in combination with pentoxyfylline and zinc against methylprednisolone, the results suggest that long term survival was similar between the two groups (66.8%, anakinra group vs. 52.8%, prednisolone group, P=0.26). Although the inflammation was shown to be reduced, and gut barrier function was improved. Targeted studies are required to identify the patients suitable for such inhibitors and combinations thereof.

**Others**

**Nutrition therapy and Immuno-nutrition**

The goals of nutrition therapy are briefly summarized in the Table 1. A detailed discussion is beyond the scope of this review.

There is also a growing realization of the benefits of immunonutrition in critical illnesses. Administration of omega-3 FAs, contained in fish oil has been shown to reduce morbidity and mortality in both, postoperative and critically ill patients, without undesired side effects. Sepsis is common in SAH patients and can develop within the first few days of the illness. Patients with SAH have insulin resistance fulfilling three dominant criteria: low high-density lipoprotein cholesterol, high triglycerides, and high fasting blood sugar. The study has shown in a recent study that omega-3 polyunsaturated FAs given intravenously for 5 days reduced the incidence and delayed the development of sepsis by improving the immunity. In fact, it was helpful in reducing the serum endotoxin levels (P<0.001) and the severity of illness. Omega-3 polyunsaturated FAs should be explored as an economical choice for the prevention of sepsis and as a source of energy. Supplementation with of energy >25 kcal (and protein >0.8 g/kg/day) has shown significant improvement in 12 month-survival in patients with alcoholic cirrhosis (Fig. 8).

**Extracorporeal liver support**

Extracorporeal liver support procedures, with potential to remove toxic and highly oxidative metabolites and circulating molecules may be of help in patients with SAH. However, artificial liver support, using different liver dialysis devices have shown limited benefits.

**Plasma exchange**

Plasma exchange has been shown to clear damage-associated molecular patterns, improve SIRS and survival in patients with acute liver failure in a large multi-centric RCT. Liver failure-related deaths were averted by plasma-exchange, both at 30 and 90 days and the therapy was superior to standard medical therapy and fractional plasma separation and adsorption in preventing organ failure and improving survival compared to the matched cohort. Apart from this, as expected plasma exchange also improved other clinical parameters and reduced the MELD scores. There is emerging data that plasma exchange, can serve as a bridge to liver transplantation in SAH patients. Though, the risk of fluid overload and infections has to be carefully weighed before taking

| Table 1. Nutritional therapy/adequacy plays an important role in recovery from severe alcoholic hepatitis |
|-----------------------------------------------|
| **Goals of nutrition support**                  |
| Adequate usable calories:                      |
| Liver regeneration                              |
| Immune restoration                              |
| Prevent hypoglycemia                            |
| Positive N2 balance, treat sarcopenia           |
| Reduce excess NH3 production:                   |
| Treat hepatic encephalopathy                    |
| Improve quality of life                         |
| Survival                                        |

Figure 8. Calorie intake of >25 kcal/kg/day demonstrates significant improvement over <25 kcal/kg/day nutrition supplementation. Adapted from Kalal et al.
patients for this therapy.

**Anti-oxidants**

Although oxidative stress has been implicated in the pathogenesis of AH, several studies have refuted benefit of N-acetylcysteine (NAC) in comparison to steroids in AH treatment. A few studies have shown short-term benefit of steroid plus NAC combination therapy with increased 1-month survival in patients with severe AH, but no increase in 6-month survival. A Cochrane review showed that the use of S-adenosyl-L-methionine is not of any help during treatment of AH. Detailed studies are required to establish the definitive role of NAC in management of severe AH. Together with oxidative stresses generated by gut microbiota investigations analyzing multiple pathological factors are required to improve our understanding, and bring in a paradigm shift in the treatment approaches for such patients.

**Granulocytapheresis**

This technique involves removal of up to 60% of activated granulocytes and monocytes from circulating blood. It has been found to be well tolerated and of some benefit in patients responding to steroids. To further elaborate the role of granulocytapheresis high quality studies are required in addition to existing case series.

**Anakinra**

Besides being compared for its anti-inflammatory potential against steroid, anakinra has also been tested for treatment of MAS, a common presentation of very sick SAH patients. The IL-1 receptor antagonist has been shown to reduce 28-day mortality in MAS patients with sepsis. Elaborate studies are required to confirm whether anakinra could be used as in combination with other anti-inflammatory drugs in steroid non-responsive patients.

**CONCLUSION**

Severe AH remains to be a disease with a high mortality, and there is an ever-growing need to find effective therapies to treat the patients early or delay mortality. From the many therapeutic options discussed above, it is unlikely that a single therapy would be an all-effective option. Given the complex molecular mechanisms varying with different stages of the disease, a combination of therapies is likely to improve efficiency. Importantly, addressing suppression of bacterial or viral infections, and hepatic inflammation, in conjunction with liver regeneration will remain the key strategy for next generation therapies.

**Authors’ contribution**

SKS and SS, both contributed to compilation of cited works, writing of review and development of figures. SKS conceptualized the manuscript.

**Conflicts of Interest**

The authors have no conflicts to disclose.

**REFERENCES**

1. Shasthry SM, Sarin SK. New treatment options for alcoholic hepatitis. World J Gastroenterol 2016;22:3892-3906.
2. André T, Tournigand C, Mineur L, Fellague-Chehra R, Flesch M, Mabro M, et al. Phase II study of an optimized 5-fluorouracil-oxaliplatin strategy (OPTIMOX2) with celecoxib in metastatic colorectal cancer: a GERCOR study. Ann Oncol 2007;18:77-81.
3. Maras JS, Das S, Sharma S, Shasthry SM, Colsch B, Junot C, et al. Baseline urine metabolic phenotype in patients with severe alcoholic hepatitis and its association with outcome. Hepatol Commun 2018;2:628-643.
4. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 2011;141:1572-1585.
5. Crabb DW, Bataller R, Chalasani NP, Kamath PS, Lucey M, Mathurin P, et al. Standard definitions and common data elements for clinical trials in patients with alcoholic hepatitis: recommendation from the NIAAA alcoholic hepatitis consortia. Gastroenterology 2016;150:785-790.
6. Shasthry SM, Sharma MK, Shasthry V, Pande A, Sarin SK. Efficacy of granulocyte colony-stimulating factor in the management of steroid-nonresponsive severe alcoholic hepatitis: a double-blind randomized controlled trial. Hepatology 2019;70:802-811.
7. Thursz MR, Richardson P, Allison M, Austin A, Bowers M, Day CP, et al. Prednisolone or pentoxifylline for alcoholic hepatitis. N Engl J Med 2015;372:1619-1628.
8. Kedarisetty CK, Anupam K, Shiv S. Insights into the role of granulocyte colony stimulating factor in severe alcoholic hepatitis. Semin Liver Dis. Forthcoming 2020.
9. Maddrey WC, Boitnott JK, Bedine MS, Weber FL Jr, Mezey E, White RI Jr. Corticosteroid therapy of alcoholic hepatitis. Gastroenterology 1978;75:193-199.
10. Duseja A, Choudhary NS, Gupta S, Dhiman RK, Chawla Y. APACHE II score is superior to SOFA, CTP and MELD in predicting the short-term mortality in patients with acute-on-chronic liver failure (ACLF).
11. Thrusz M, Morgan TR. Treatment of severe alcoholic hepatitis. Gastroenterology 2016;150:1823-1834.
12. Louvet A, Naveau S, Abdelnour M, Ramond MJ, Diaz E, Fartoux L, et al. The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. Hepatology 2007;45:1348-1354.
13. Mathurin P, O'Grady J, Carithers RL, Phillips M, Louvet A, Mendenhall CL, et al. Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis: meta-analysis of individual patient data. Gut 2011;60:255-260.
14. Mathurin P, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, et al. Early liver transplantation for severe alcoholic hepatitis. N Engl J Med 2011;365:1790-1800.
15. Dao A, Ranganekar AS. Steroids for severe alcoholic hepatitis: more risk than reward? Clin Liv Dis 2018;12:151-153.
16. Louvet A, Wartel F, Castel H, Dharnassy S, Hollebecque A, Canva-Delcambre V, et al. Infection in patients with severe alcoholic hepatitis treated with steroids: early response to therapy is the key factor. Gastroenterology 2009;137:541-548.
17. Garcia-Saenz-de-Sicilia M, Duvoor C, Altamirano J, Chavez-Araujo R, Prado V, de Lourdes Candolo-Martineili A, et al. A day-4 Lille model predicts response to corticosteroids and mortality in severe alcoholic hepatitis. Am J Gastroenterol 2017;112:306-315.
18. Shasthry SM, Rastogi A, Bihari C, Vijayaraghavan R, Arora V, Sharma MK, et al. Histological activity score on baseline liver biopsy can predict non-response to steroids in patients with severe alcoholic hepatitis. Virchows Arch 2018;472:667-675.
19. Sukriti S, Maras JS, Bihari C, Das S, Vyas AK, Sharma S, et al. Micrvesicles in hepatic and peripheral vein can predict nonresponse to corticosteroid therapy in severe alcoholic hepatitis. Ailment Pharmacol Ther 2018;47:1151-1161.
20. Sharma S, Maras JS, Das S, Hussain S, Mishra AK, Shasthry SM, et al. Pre-therapy liver transcriptome landscape in Indian and French patients with severe alcoholic hepatitis and steroid responsiveness. Sci Rep 2017;7:6816.
21. Petta I, Dejager L, Ballegeer M, Lievens S, Tavernier J, De Bosscher K, et al. The interactome of the glucocorticoid receptor and its influence on the actions of glucocorticoids in combattting inflammatory and infectious diseases. Microbiol Mol Biol Rev 2016;80:495-522.
22. Das S, Maras JS, Hussain MS, Sharma S, David P, Sukriti S, et al. Hyperoxidized albumin modulates neutrophils to induce oxidative stress and inflammation in severe alcoholic hepatitis. Hepatology 2017;65:631-646.
23. Tay J, Levesque JP, Winkler IG. Cellular players of hematopoietic stem cell mobilization in the bone marrow niche. Int J Hematol 2017;105:129-140.
24. Scadden D, Silberstein L. Hematopoietic microenvironment. In: Hoffman R, Benz E, Weitz J, eds. Hematology. 7th ed. Philadelphia: Elsevier, 2018:119-126.
25. Karakkei E, Moreno C, Gustot T. Infections in severe alcoholic hepatitis. Ann Gastroenterol 2017;30:152-160.
26. Mookerjee RP, Stadlbauer V, Lidder S, Wright GA, Hodges SJ, Davies NA, et al. Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome. Hepatology 2007;46:831-840.
27. Artru F, Bou Saleh M, Maggiotto F, Lassailly G, Ningarhari M, Demaret J, et al. IL-33/ST2 pathway regulates neutrophil migration and predicts outcome in patients with severe alcoholic hepatitis. J Hepatol 2020;72:1052-1061.
28. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hepatol Int 2019;13:353-390.
29. Khanam A, Trehanpati N, Garg V, Kumar C, Garg H, Sharma BC, et al. Altered frequencies of dendritic cells and IFN-γ-secreting T cells with granulocyte colony-stimulating factor (G-CSF) therapy in acute-on-chronic liver failure. Liver Int 2014;34:505-513.
30. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 2006;124:407-421.
31. Piscaglia AC, Shupe TD, Oh SH, Gasbarrini A, Petersen BE. Granulocyte-colony stimulating factor promotes liver repair and induces oval cell migration and proliferation in rats. Gastroenterology 2007;133:619-631.
32. Garg V, Garg H, Khan A, Trehanpati N, Kumar A, Sharma BC, et al. Granulocyte colony-stimulating factor mobilizes CD34+ cells and improves survival of patients with acute-on-chronic liver failure. Gastroenterology 2012;142:505-512.e1.
33. Singh V, Sharma AK, Narasimhan RL, Bhatta A, Sharma N, Sharma R. Granulocyte colony-stimulating factor in severe alcoholic hepatitis: a randomized pilot study. Am J Gastroenterol 2014;109:1417-1423.
34. Prajapati R, Arora A, Sharma P, Bansal N, Singh V, Kumar A. Granulocyte colony-stimulating factor improves survival of patients with decompensated cirrhosis: a randomized-controlled trial. Eur J Gastroenterol Hepatol 2017;29:448-455.
35. Marot A, Singal AK, Moreno C, Deltenre P. Granulocyte colony-stimulating factor for alcoholic hepatitis: a systematic review and meta-analysis of randomised controlled trials. JHEP Rep 2020;2:100139.
36. Gameiro J, Agapito Fonseca J, Jorge S, Lopes JA. Acute kidney injury definition and diagnosis: a narrative review. J Clin Med 2018;7:307.
37. Bihari C, Anand L, Rooge S, Kumar D, Saxena P, Shubham S, et al. Bone marrow stem cells and their niche components are adversely affected in advanced cirrhosis of the liver. Hepatology 2016;64:1273-1288.
38. Anand L, Bihari C, Kedarissetty CK, Rooge SB, Kumar D, Shubham S,
et al. Early cirrhosis and a preserved bone marrow niche favour regenerative response to growth factors in decompensated cirrhosis. Liver Int 2019;39:115-126.

39. Liu Y, Chen K, Li F, Gu Z, Liu Q, He L, et al. Probiotic Lactobacillus rhamnosus GG prevents liver fibrosis through inhibiting hepatic bile acid synthesis and enhancing bile acid excretion in mice. Hepatology 2020;71:2050-2066.

40. Sarin SK, Pande A, Schnabl B. Microbiome as a therapeutic target in alcohol-related liver disease. J Hepatol 2019;70:260-272.

41. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Nat Immunol 2013;14:685-690.

42. Llopis M, Cassard AM, Wrzosek L, Boschat L, Bruneau A, Ferre G, et al. Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. Gut 2016;65:830-839.

43. Xie G, Zhong W, Zheng X, Li Q, Qiu Y, Li H, et al. Chronic ethanol consumption alters mammalian gastrointestinal content metabolites. J Proteome Res 2013;12:3297-3306.

44. Chen P, Torralba M, Tan J, Embree M, Zengler K, Stärkel P, et al. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver injury in mice. Gastroenterology 2015;148:203-214.e16.

45. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. Proc Natl Acad Sci U S A 2006;103:3920-3925.

46. Hartmann P, Hochrath K, Horvath A, Chen P, Seebauer CT, Llorente C, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. Hepatology 2018;67:2150-2166.

47. Philips CA, Pande A, Shasthry SM, Jamwal KD, Khillan V, Chandel SS, et al. Healthy donor fecal microbiota transplantation in steroid-ineligible severe alcoholic hepatitis: a pilot study. Clin Gastroenterol Hepatol 2017;15:600-602.

48. Hartmann P, Hochrath K, Horvath A, Chen P, Seebauer CT, Llorente C, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. Hepatology 2018;67:2150-2166.

49. Kelly CR, Kahn S, Kashyap L, Laine R, Rubin D, Atreja A, et al. Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. Gastroenterology 2015;149:223-237.

50. Defilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Hunter MH, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. N Engl J Med 2019;381:2043-2050.

51. Szabo G, Mitchell MC, McClain CJ, Dasarathy S, McCullough AJ, Nagy L, et al. IL-1β Receptor antagonist in combination with pentoxifylline and zinc for severe alcoholic hepatitis: a multicenter randomized double-blind placebo-controlled clinical trial. Hepatology 2018;68:1444A-1471A.

52. Zirnheld KH, Warner DR, Warner JB, Hardesty JE, McClain CJ, Kirkpatrick RP. Dietary fatty acids and bioactive fatty acid metabolites in alcoholic liver disease. Liv Res 2019;3:206-217.

53. Oral abstracts (abstracts 1–263). Hepatology 2017;66:1-148.

54. Anand L, Benjamin J, Kumar A, David P, Vyas A, Pandit V, et al. Short-term infusion of soybean oil based intravenous lipid emulsion is safe in patients with acute-on-chronic liver failure (ACLF), improves nitrogen balance and preserves immune functions: 2088. Hepatology 2015;62:197A.

55. Kalal C, Benjamin J, Shasthry V, Philips CA, Vyas TS, Premkumar M, et al. Effect of long term aggressive nutritional therapy on survival in patients with alcoholic liver cirrhosis-interim analysis of a randomized controlled trial. Hepatology 2016;64 Suppl 1:705A-706A.

56. Maiwall R, Maras JS, Nayak SL, Sarin SK. Liver dialysis in acute-on-chronic liver failure: current and future perspectives. Hepatol Int 2014;8 Suppl 2:505-513.

57. Vanare S, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, et al. Extracorporeal albumin dialysis with the molecular adsorbent re-circulating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology 2013;57:1153-1162.

58. Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, et al. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology 2012;142:782-789.e3.

59. Maiwall R, Choudhury A, Duan Z, Chen Y, Hu J, Ghazinour HL, et al. Therapeutic plasma-exchange improves systemic inflammation and survival in patients with acute on chronic liver failure. J Hepatol 2018;68:S45.

60. Phillips M, Curtis H, Portmann B, Donaldson N, Bomford A, O’Grady J. Antioxidants versus corticosteroids in the treatment of severe alcoholic hepatitis--a randomised clinical trial. J Hepatology 2006;44:784-790.

61. Nguyen-Khac E, Thevenot T, Piquet MA, Benferhat S, Goria O, Chatelain D, et al. Glucocorticoids plus N-acetylcysteine in severe alcoholic hepatitis. N Engl J Med 2011;365:1781-1789.

62. Kamimura K, Imai M, Sakamaki A, Mori S, Kobayashi M, Mizuno K, et al. Extracorporeal albumin dialysis with the molecular adsorbent re-circulating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology 2013;57:1153-1162.

63. Morris JM, Dickson S, Neilson M, Hodgins P, Forrest EH. Granulocytapheresis in the treatment of severe alcoholic hepatitis: a case series. Eur J Gastroenterol Hepatol 2010;22:457-460.

64. Kamimura K, Imai M, Sakamaki A, Mori S, Kobayashi M, Mizuno K, et al. Granulocytapheresis for the treatment of severe alcoholic hepatitis: a case series and literature review. Dig Dis Sci 2014;59:482-488.

65. Schulte G, Grom AA. Pathogenesis of macrophage activation syndrome and potential for cytokine-directed therapies. Annu Rev Med 2015;66:145-159.

66. Shakoori B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA.
Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of macrophage activation syndrome: reanalysis of a prior phase III trial. Crit Care Med 2016;44:275-281.

Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. Nat Rev Gastroenterol Hepatol 2016;13:131-149.