Acute on Chronic Liver Failure: Prognosis and Biomarkers:

- ***How improved pathological insight of ACLF has informed new biological markers which, with future validation, may help to stratify and monitor response to therapy***

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From early descriptions of the syndrome some 13 years ago [Jalan and Williams Blood Purif 2002; 20(3):252-61] extra-hepatic organ failure and a systemic inflammatory response were considered important factors underpinning the evolution of ACLF and thereby entities to include in prognostic assessment. Scrolling forward a decade, and with a much clearer definition of the syndrome [Moreau et al, Gastro 2013; 144:1426-37], it is now recognized that ACLF is a very dynamic syndrome where up to 50% of patients may undergo complete resolution from the decompensation event. With this comes the understanding that the short-term mortality is most accurately determined by the evolution of the clinical course from initial presentation to final ACLF grade obtained (usually within 7 days of presentation). [Gustot et al Hepatol 2015;62(1): 243-52].

Scoring systems for ACLF:

Whilst conventional scoring systems such as the Child Pugh and Model for End-Stage Liver Disease (MELD) scores and their variants have been used to define prognosis in cirrhosis and determine the utility of transplantation, they are limited in their prognostic accuracy in ACLF due to a failure to incorporate two central prognostic determinants, namely, extra-hepatic organ failures and measures of systemic inflammation. These two entities underpin the pathophysiological basis of the syndrome of ACLF. The recent large European multi-center prospective clinical study evaluating over 1300 hospitalized patients with cirrhosis complications- the CANONIC study- (Moreau R et al, Gastroenterology. 2013;144:1426-37), has helped define the phenotypes of ACLF patients. However, importantly, the study also helped assess the currently available prognostic scoring systems and enabled the development and validation of novel scoring systems for the prognosis of patients with ACLF (Jalan R et al, J Hepatol. 2014;61:1038-47) and acute decompensation (AD) (Jalan R et al, J Hepatol. 2015;62:831-40). Thus several grades of ACLF were classified using a modified sequential organ failure assessment (SOFA) score, evaluating liver, kidney, brain, coagulation, circulation and respiratory function (table 1). ACLF grades (1-3) were found to be highly predictive of mortality with significantly different outcomes. Response to treatment of ACLF patients may be monitored by daily calculation of the CLIF Consortium ACLF score (CLIF-C ACLFs), incorporating the CLIF-OF score, age and log-transformed white cell count (reflecting systemic inflammation). Ultimately, resolution of ACLF is the most important determinant of short and medium term mortality and the CLIF-C scores provide an objective measure of this. The CLIF-C ACLFs has since been independently validated with proven superior prognostic accuracy for ACLF compared to conventional scoring systems such as MELD, and Child-Pugh scores, and of note, provides a dynamic score to assess evolution to a potentially severe early course. It follows that such a scoring system facilitates a treatment algorithm whereby, patients with high CLIF-C ACLF scores may be considered for liver transplantation. If ineligible for transplantation, without a demonstrable treatment response in the score with standard of care therapy by days

3-7, consideration should be given to placing ceilings of care management. The scores are freely available on the CLIF Consortium website and also as an app that is downloadable on any mobile platform (ACLF Calculator, Cyberliver, UK).

What remains unclear is whether such scoring systems, which are at best about 75 percent accurate, apply effectively to all etiologies of liver disease, with an increased appreciation of an 'Asian' viral hepatitis B predominant phenotype of ACLF where there is largely a hepatic hit, compared to a 'Western' alcohol and viral hepatitis C predominant ACLF, where extrahepatic disease is commonly described. The heterogeneity of existing definitions of ACLF between Asia-Pacific [Garg et al, Dig Liver Dis 2012; 44(2): 166-71] and Europe-North America [Olson and Kamath Curr Opin Crit Care 2011; 17(2):165-9] have added to the lack of clarity for the importance of precipitating factors, the key role of systemic inflammation and the liver-centric focus of the acute insult. Moreover, scoring systems applied to cirrhosis decompensation such as MELD are believed to have limitations in the context of an inflammatory syndrome such as ACLF, largely from the limitations of creatinine as a weighted parameter in this setting. In addition, many aetiology specific scoring systems exist, especially for alcoholic hepatitis, such as the Maddrey score, Lille score and Glasgow scores. However, how the CLIF-C score compares with these other scores requires assessment in prospective future studies. Thus unanswered questions remain as to (i) whether there are different pathophysiological processes that underlie the different etiologies and precipitant expressions of ACLF; (ii) In the absence of gold standard markers of prognosis to render one 'scoring system' superior to another, are other markers of pathophysiology more apt to define outcome?. It follows that a more detailed characterization of factors reflecting inflammation, nature of liver injury and markers of extra-hepatic organ function in these different settings, may be more effective as biomarkers of prognosis. This review aims to explore these factors in more detail.

Markers of outcome in ACLF:

The main rationale for pursuing a reliable biomarker in ACLF is to help with early detection of evolution of the syndrome in patients likely to evolve to a severe prognostic group. In such patients, timely intensive intervention may facilitate reversal of the process or/and improve survival, and define those patients who may benefit from fast-track listing for liver transplantation. Conversely, in patients defined as too advanced with futility as mortality approaches 75-100 percent, there is a health economic argument that costly interventions such as liver support should be avoided in place of end-of-life supportive care. As stated above, deploying the CLIF-Organ Failure score within the first week of evolution of decompensation, enables a dynamic score to help guide treatment allocation since this has been suggested to best stratify patients by their clinical and prognostic significance.[Jalan J Hep 2014; 61:1038-47] An observation worthy of note when deploying this score in a dynamic way is that precipitating events such as alcohol use and presence of infection do not appear to have a statistically significant bearing on 28-day or 90-day mortality.

Oxidative Stress Factors:

Inflammation and oxidative stress are believed to be key pathophysiological processes in the development of ACLF [Jalan Curr Opin Crit Care 2011;17(2): 152]. Indeed, studies have explored the role of inexpensive clinical markers such as CRP in the context of acute cirrhosis decompensation, and shown them to improve upon the prognostic utility of MELD score [Di Martino V et al, Liver Transpl 2015; 21(6) 753-60], highlighting the role of inflammation in this process. However, the application of different cut-off values for CRP across institutions limits interpretation of such data. Similarly, lack of resolution of inflammatory response is implied by the incorporation of white blood cell count into the CLIF-C ACLF score. However, these global systemic inflammatory markers still fail to indicate the degree of local hepatic and organ specific injury. To this end, small studies such as by Cai et al [Clin Res hepatol and Gastroenterol 2016; 40(1): 41-50] in patients with hepatitis B related ACLF reflect the impact of hepatic oxidative stress, denoted by advanced oxidation protein products, binding to their receptors such as RAGE, to trigger hepatocyte apoptosis and necrosis. Their data show that dynamic plasma levels of S100A12 and sRAGE could define the group of non-survivors. From the pathophysiological perspective, increased oxidation products are likely to drive activation of inflammatory cells such as neutrophils and monocytes, with the resulting cytokine cascade promoting further organ injury. This is reflected by increased generation of HMGB1 in non-survivors, which serves as a marker of pro-inflammatory drive. [Sha Y et al, J Immunol 2008; 180(4): 2531-7] Moreover, during inflammation, oxidation of albumin with the development of non-mercaptalbumin 1 and 2 has been shown to directly impact on binding quality of albumin with prognostic implication for 30- and 90-day survival. [Oettl K et al, J Hepatol 2013; 59(5): 978-83]. Oxidation of albumin also leads to the generation of ischaemia modified albumin, which may also serve as a prognostic index in ACLF [Jalan et al, Hepatology 2009; 50(2); 555-64] and provides some rationale for the benefits of albumin infusion therapy in ACLF.

Markers of Cell Death:

The impact of cell death as a consequence of hepatic inflammation has also been explored as potential biomarkers in ACLF. Cao and colleagues studied the caspase cleaved neo-epitope of cyto-keratin18 (M-30 antigen) indicating hepatocyte apoptotic death and the intact K-18 variants (M-65 antigen) reflecting total death of hepatocytes, in patients with hepatitis B ACLF.[Cao Z et al. Sci Rep 2015; 18(5):14240] The ratio of M30/M65 was used to indicate apoptosis rate, which at basal levels was considerably higher in patients with ACLF compared to chronic HBV cirrhosis. Of interest, they found that dynamic measurement of M30/M65 as a percentage of day 1 values of M30 antigen, increased in survivors, suggesting an important role of apoptosis in the recovery of HBV-ACLF. Another study assessing M-30 and M-65 in plasma from a mixed UK population presenting with ACLF showed M-30 to be significantly elevated in non-survivors and in those patients with extra-hepatic failure. [Adebayo D et al, Liver Int 2015;35(12)2564-74] This questions whether such markers of apoptosis can also

be used as targets for therapy. However, whilst excessive hepatocyte death is undoubtedly a key event in the development of ACLF, it raises new questions over the importance of which mode of cell death predominates as ACLF progresses. Hepatocyte regeneration may well necessitate activation of caspases [Ben Moshe T et al Hepatology 2007; 45(4):1014-24], but the exact balance of apoptosis versus necrosis as mode of cell death in ACLF clearly requires further study. Furthermore, as plasma measures of M30 antigen are made increasing available, it raises the possibility for an early prognostic biomarker in patients with ACLF which warrants further evaluation across different precipitants of ACLF. Cao et al did demonstrate that measures of cell death when added to traditional prognostic scores such as MELD-Na, did provide superior prognostic utility. Again, the addition of such markers to scores such as the CLIF-C ACLF score requires further study.

Immune dysfunction and Gut Dysbiosis:

With advancing cirrhosis or/and an acute hepatic hit, there is significant loss of gut barrier integrity and increased bacterial translocation with pathogen associated molecular patterns (PAMPs) triggering immune activation. [Mencin A et al, Gut 2009; 58(5):704-20] Current theory suggests that in ACLF, there is relative immune dysregulation with loss of functional adaptive and innate immune responses, resulting in an immune-paretic state and increased incidence of sepsis. [Gustot T et al, Hepatology 2015; 62(1): 243-52] Recent data suggests that in ACLF patients there are increased numbers of monocytes and macrophages expressing the receptor tyrosine kinase MERTK+, which in turn down regulates innate immune responses to microbial challenge. [Bernsmeier C et al, Gastro 2015; 148(3): 603-15] MERTK+ cell expression correlated with the severity of hepatic and extrahepatic disease and systemic inflammatory responses and reduced the response of cultured monocytes to lipopolysaccharide. This follows on from earlier literature suggesting that monocyte HLA-DR expression was pathologically down regulated in decompensated cirrhosis [Wasmuth HE J Hepatol 2005; 42(2): 195-201]. Thus immune phenotyping studies in cohorts of ACLF may lead to characterization of an immune ‘fingerprint’ that best defines a group with a high risk of sepsis and mortality, and in need of early intervention with novel therapies such as potential use of MERTK inhibitors.

Neutrophil dysfunction with high oxidative burst function and reduced phagocytosis has also been characterized in decompensated cirrhosis and is most marked in those with organ failure and is associated with increased mortality. [Mookerjee Hepatology 2007; 46(3): 831-40] It is believed that this in part contributes to further hepatic injury through the liberation of increased oxidant species and as a consequence, reduced cellular ATP reserves contribute to impaired ability to phagocytose pathogens and consequent immune dysfunction.

Recently, Ariza and colleagues [J Hep 2016- ePub] have studied neutrophil gelatinase-associated lipocalin (NGAL) in patients with ACLF. They show that the *LCN2* gene is markedly upregulated in ACLF livers, and its product, NGAL, is increased in the urine and plasma of these patients. Importantly, they show that the predictive utility of NGAL remains even after adjustment is made for urinary function, given that NGAL levels are influenced by glomerular filtration. They found that urinary NGAL improved significantly the accuracy of MELD in predicting outcome. Urinary NGAL was shown to correlate with markers of liver failure (Bilirubin and INR) and also with measures of systemic inflammation (Interleukin-6). They propose that given the high levels of *LCN2* gene in ACLF, urinary NGAL could be a reliable biomarker of ACLF. Clearly, this requires validation across different precipitants of ACLF and it would be interesting to note whether Urinary NGAL adds further to the dynamic predictive utility of the CLIF-C ACLF score.

A further interesting approach has been to consider phenotyping the gut microbiome in ACLF patients, to assess the level of gut dysbiosis. A study by Chen et al in a mixed ACLF cohort, though the predominant etiology was hepatitis B, showed a marked decrease in relative abundance of *Bacteroidetes* and conversely, an increase in *Firmicutes*. [Chen et al, J Gastroenterol Hepatol 2015; 30(9): 1429-37] At the family level of bacteria, the *Ruminococcaceae* and *Lachnospiraceae* were significantly decreased in ACLF, whilst *Streptococcaceae, Pasteurellaceae* and *Enterococcaceae* were significantly increased. *Pasteurellaceae* was also noted to be significantly elevated in non-survivors whilst low levels of *Lachnospiraceae* were found in patients that died. Furtehrmore, IL-6 levels were noted to be negatively correlated with *Ruminococcaceae* and *Lachnospiraceae.* A further important finding in this study was that whilst the gut microbiota reached a new equilibrium following onset of ACLF, the use of antibiotics in management of these patients had only a limited impact on the composition of the gut microbiota. The short-term stability of the gut microbiota after onset of ACLF does suggest that such characterization might also be of diagnostic and prognostic benefit but clearly larger studies across different liver disease etiologies are needed to understand whether such microbial patterns can be generalized to all ACLF cases, and validated for prognostic utility.

Measures reflecting Organ Failure:

Haemodynamic derangement: ACLF has been described as aggravating portal hypertension and indeed it is well established that in patients with for example alcoholic hepatitis precipitated ACLF, the inflammatory response significantly increases intrahepatic resistance Indeed, there is a good correlation between histological grade of inflammation in ACLF and severity of portal hypertension [Mookerjee J Hepatol 2011; 55(5): 1103-11] Recently, Garg and co-workers have shown that baseline HVPG is a predictor of mortality in patients with ACLF and that the high early rise in portal pressure, may predispose to increased risk of variceal bleeding. [Garg et al, J Gastroenterol Hepatol, 2013; 28(8): 1361-7] Moreover, as a reflection of aggravation of the systemic hyperdynamic response, the presence of

hyponatremia in ACLF was found to have an independent effect on 90-day survival after adjusting for other potential confounders. Patients with hyponatremia and ACLF had a three-month transplant-free survival of only 35.8% compared to 58.7% in those with ACLF without hyponatremia (P <0.001).[Cardenas et al, Crit Care 2014; 18(6): 700]

Most recently, emerging data has focused on the glycopeptide, Copeptin, which is released from the neurohypophysis along with vasopressin (AVP). Solà and colleagues show Copeptin levels in patients with decompensated cirrhosis had a significant direct correlation with MELD score, AVP levels, endogenous vasoconstrictor activation and kidney function parameters. Copeptin levels were most elevated in non-survivors and in patients that developed decompensation.[Solà E et al, J Hepatol 2016 epub] It follows that neuro-hormonal markers such as Copeptin, renin and metabolites of norepinephrine might serve as indicators of systemic activation of such important regulatory pathways in the context of ACLF and may add additional prognostic utility to standard physiological measures such as mean arterial pressure and HVPG, when incorporated in prognostic scores. Clearly this requires further study and validation. Biomarkers of microvascular dysfunction have also been implicated in ACLF, associated with the severity of inflammatory response. Large von Willebrand Factor complexes have been described in patients with alcoholic hepatitis through deficiency of ADAMTS13 and thought in part to contribute to microcirculatory dysfunction and multi-organ failure.[Matsuyama et al, Alcohol Clin Exp Res 2007; 31(1-Suppl) S27-35], and provide prognostic utility in the context of sepsis [Reuken P et al, Liver Int 2015; 35(1): 37-45].

Along a similar vein, asymmetric dimethylarginine, a potent endothelial NOS regulator, has been shown to be significantly elevated in liver failure in the context of hepatic inflammation [Mookerjee Liver Transpl 2007; 13(3): 400-5] and importantly, has prognostic utility in patients with ACLF secondary to alcoholic hepatitis [Mookerjee Hepatol 2007]. A subsequent study focused on a product of both ADMA and its stereo-isomer Symmetric Dimethylarginine (SDMA), which is believed to have impact on expression of nitric oxide synthase but also to be a sensitive indicator of renal function. In a study of 52 patients with acute decompensation of cirrhosis, the comibined dimethylarginines predicted development of ACLF and also 28-day mortality. The addition of ischaemia modified albumin ratio (IMAR) to the dimethylarginine score enabled superior predictive utility for mortality with an area under receiver operator curve of 0.9 (CI: 0.82-0.99; sensitivity 92% and specificity 80%) Figure 1 [Mookerjee et al, Hepatology 853, vol 46 (4) Suppl 1, 2007, 615A] An on-going study which has been evaluating the role of this marker in decompensated cirrhosis and ACLF is soon to be reported.

Renal Failure: Given the findings of the CANNONIC study and the important prognostic role that development of renal failure plays in defining the first stage of ACLF [Moreau Gastro 2013; 144(7): 1426-37], an accurate marker of renal functional reserve is clearly required. As stated above, creatinine has significant short comings

as a renal function marker in advanced cirrhosis and is perhaps in part the reason for why MELD has less apparent utility in ACLF compared to organ failure scores. Wan and colleagues assessed serum cystatin C in patients with HBV induced ACLF and were able to show that it predicted onset of acute kidney injury in ACLF, even when patients demonstrated normal serum creatinine range levels.[Wan et al, World J Gastroenterol 2013;19(48): 9432-8]. More recently, Ariza et al analyzed a panel of urinary biomarkers and showed that NGAL, in addition to osteopontin and trefoil-factor-3 served as good markers for the development of ACLF and outcome. [Ariza X et al, PLoS One 2015; 10(6): e0128145] Clearly, such findings require further validation in larger studies and in different etiologies but challenge the more standard criteria used in scores such as MELD.

In summary, a number of markers of oxidative and cellular injury are emerging, along with factors that reflect organ specific injury. Whilst some such as urinary NGAL have been tested in large patient groups, most of these 'biomarkers' still require extensive validation in relevant, heterogenous populations of ACLF. Future studies, beyond just validation of the individual biomarkers, will focus on developing a composite marker, to be used in conjunction with clinical scoring systems that best predict response to early intervention in ACLF (first week of admission) and demonstrate the best prognostic utility. Further development will require refinement of biological assays that will allow point-of-care testing ideally in remote care settings, such that home monitoring of cirrhosis can become not just an aspiration but a reality to better serve this patient population.

Figure 1:

This 3-dimensional representation of the components of the DASIMAR score, namely, ADMA, SDMA and IMAR are shown for patients with compensated alcoholic cirrhosis (green); decompensated alcoholic cirrhosis who survive after recovering from their acute decompensation (blue); and those with ACLF as a consequence of alcoholic hepatitis (red). It is evident form the figure that those patients with acute decompensation, have higher ADMA and SDMA values, then the compensated cirrhosis group. However, the highest values for ADMA, SDMA and especially IMAR, are amongst the non-surviving group of alcoholic hepatitis patients following their acute decompensation.

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