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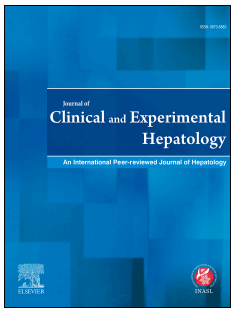

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Loretta Jophlin developed the initial draft and searched the literature for the review.

AKS reviewed the draft and added intellectual content to the review.

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Liver biopsy in patients with alcohol-associated liver disease with acute on chronic liver failure

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Abstract

Patients with alcohol-associated liver disease may develop severe forms of presentation of acute on chronic liver failure, with high risk for short-term mortality. Alcoholic hepatitis should be suspected among patients with alcohol-associated liver disease who present with acute on chronic liver failure. In this review, we discuss the need and feasibility of liver biopsy in the diagnosis of alcoholic hepatitis and predicting its prognosis among decompensated patients with alcohol-associated liver disease and acute on chronic liver failure.

Acute on chronic liver failure (ACLF) is a unique syndrome with sudden onset of hepatic decompensation among patients with chronic liver disease and/or cirrhosis. The criteria for diagnosis of ACLF are heterogeneous across the world, [1, 2] without consensus among the set of criteria proposed by different consortia (Table 1). However, the common factor connecting these definitions is a risk for high mortality irrespective of whichever criteria are used to define ACLF. For this review, we will exclude the ACLF definition proposed by the Chinese Group on the Study of Severe Hepatitis B as it focuses on hepatitis B virus induced ACLF, and the criteria proposed by the North American Consortium for the Study of End-stage Liver Disease, as this definition only includes ACLF patients precipitated by infection. The Asia-Pacific ACLF Research Consortium (AARC) defines ACLF with the following set of criteria: serum bilirubin ≥ 5 mg/dL and INR ≥ 1.5 and development of clinical ascites and/or encephalopathy within 28 days in an individual with chronic liver disease or cirrhosis [3]. The EASL-Chronic Liver Failure (CLIF) consortium defines ACLF in the presence of one or more of the six organ failures in an individual with cirrhosis [1]. A major difference is that EASL-CLIF proposes that the chronic component of ACLF be manifested by cirrhosis whereas the AARC criteria includes individuals with chronic liver disease with and without cirrhosis. In the EASL-CLIF CANONIC trial, “active alcoholism” defined in individuals with active alcohol use within 3 months of presentation, was identified in 23% of subjects with ACLF and was strongly associated with ACLF severity [4].

Rationale in favor of liver biopsy for the detection of AH as a precipitant of ACLF

ACLF in patients with alcohol-associated liver disease (ALD) could be due to acute alcoholic hepatitis (AH) superimposed on underlying advanced fibrosis or cirrhosis which is commonly present in these patients,[5, 6] or be due to non-AH precipitants such as bacterial infection, gastrointestinal bleeding, drug induced liver injury or toxic encephalopathy.[2, 7-9] Further, in a recent prospective study from the EASL-CLIF consortium, AH and/or bacterial infection were

the most common precipitants of ACLF, and were present in about 96% of patients with cirrhosis.[10]

The decision to make an accurate diagnosis of AH in an ACLF patient depends on whether the patient is eligible for specific AH treatment or for clinical trial enrollment [11-13]. Corticosteroids are the only available specific pharmacological treatment for AH, with a short-term survival benefit of only about 50% for one month.[13, 14] Further, many severe AH patients may be ineligible for this therapy due to presence of contraindications,[15] and even in eligible patients the response is observed in only 50-60% cases and cannot be predicted before initiation of treatment.[16] For example, a patient with ACLF in the ICU on mechanical ventilation, pressor, and dialysis would not be a candidate for corticosteroids or for enrollment in clinical trials evaluating newer therapeutic targets. In such cases, there is no need to obtain a liver biopsy to make a diagnosis of AH. In another scenario, a patient with coagulation failure and early encephalopathy not requiring ICU-level care may benefit from corticosteroid therapy if there are no contraindications. Such a patient would benefit from making an accurate diagnosis of AH. Among patients ineligible for steroids or clinical trials under consideration for early liver transplantation via an exception pathway (i.e. not requiring minimum six months of abstinence), the treating physician may utilize liver biopsy for the diagnosis of AH on a case-by case basis. For example, within the EASL-CLIF CANONIC prospective study, liver biopsy was performed in a minority, which limited accurate estimation of the true prevalence of AH in this study [4].

Defining Alcoholic Hepatitis

The definite diagnosis of AH requires a liver biopsy.[5, 17] As it is invasive with risk of complications in patients with AH, especially those with ascites and/or coagulopathy, [18, 19] biopsy is not performed by most centers and providers.[15, 20] As such, the National Institute on Alcoholism and Alcohol Abuse (NIAAA) funded consortia using prospectively enrolled patients with AH developed criteria for the clinical diagnosis of AH.[11]. These criteria are:

onset of jaundice within 8 weeks (60 days) of last alcohol use, daily harmful alcohol use for 6 months or more, serum bilirubin (total) > 3.0 mg/dL, aspartate aminotransferase and alanine aminotransferase > 50 and <400 IU/L with aspartate aminotransferase/alanine aminotransferase > 1.5, and exclusion of other causes of liver disease including cholangitis and superimposed hepatocellular carcinoma. Of these criteria, the most challenging is inaccuracy and underreporting by the patient on alcohol consumption due to the social stigma associated with alcohol use disorder (AUD) and change in mental status due to alcohol withdrawal and/or hepatic encephalopathy.[21, 22]

Self-reported information on alcohol use should be cross checked with the family members, close friends, and with other health care providers.[13, 23] In this regard, biomarkers of alcohol consumption can provide useful information and supplement the self-reported data.[24] Serum gamma-glutamyl transferase, AST, mean corpuscular volume, and carbohydrate-deficient transferrin are easily available but have low specificity to identify alcohol use.[24-26] Emerging biomarkers of alcohol metabolism like ethyl glucuronide (EtG) and phosphatidylethanol (PEth) are more accurate. Estimation of urinary level of EtG, a non-volatile water-soluble alcohol metabolite, can identify alcohol use over the last four days with a sensitivity of 62-89% and a specificity of 93-99%.[13, 24-27] PEth is a phospholipid metabolite of alcohol in the membrane of red blood cells, and can identify use of alcohol over the previous four weeks with a sensitivity of 90-99% and specificity of 100%.[24, 26, 27] Given the rapid onset of ACLF (typically <4 weeks) and last alcohol drink required within 8 weeks of presentation, PEth may be cautiously used in these patients to determine if alcohol may be the precipitant. [28, 29] Studies are needed to examine the utility of PEth for diagnosis of AH. Another challenge in clinical diagnosis of AH is in excluding concomitant other causes of liver disease, [30] especially chronic hepatitis C virus infection, which may be present in up to 25% of patients with chronic liver disease and/or cirrhosis.[31-34] It should be recognized that ALD and AH can occur at a lower amount of alcohol use in the presence of hepatitis C or B virus infections or

other concomitant comorbidities such as obesity, type 2 diabetes mellitus, metabolic syndrome, or another chronic liver disease.[35]

When all these criteria are met, it is reasonable to make a probable diagnosis of AH for specific pharmacological treatment with corticosteroids and/or inclusion in clinical trials. As the clinical diagnosis of AH may be inaccurate in 4-46% cases especially when one or more of these criteria are not met,[7, 36, 37] such patients with possible AH should have a liver biopsy for making a diagnosis of definite AH. A liver biopsy may also be obtained depending on center or provider discretion among patients who qualify for a diagnosis of probable AH, and meeting all the clinical criteria (**Figure 1**).

Accuracy of clinical diagnosis of AH

In a pooled dataset from 11 randomized clinical trials recruiting biopsy-proven AH patients, the accuracy of the clinical diagnosis of AH was 84.5%, and the accuracy increased to 96% among patients with bilirubin >4.7 mg/dL [38]. In a post-hoc analysis of the largest randomized controlled trial in AH, the STOPAH trial, clinical diagnosis of AH was accurate in 80% of patients with severe AH (MELD score>20). This accuracy of clinical diagnosis increased to 100% in patients undergoing liver biopsy examination before administration of corticosteroids and baseline MELD score of ≥25 [39]. In another study, white cell and platelet counts were helpful in making a more accurate diagnosis of AH. [40, 41]. For example, in a study on 68 patients with ALD and receiving liver biopsy (35 with AH on liver biopsy), white cell count <5.95 and platelet count <86 provided a 83% negative predictive value for excluding the diagnosis of AH, and white cell count of >10.95 and platelet count of >147.5 had a 100% positive predictive value in diagnosis of AH.[41]

Liver biopsy: **technique and findings**

A trans jugular approach is recommended to obtain the liver tissue, especially among patients with ascites and/or coagulopathy.[5, 13, 17, 19, 42] Safety of liver biopsy in patients with

decompensated liver disease especially those with ACLF is also a concern. In an early report, open liver biopsy performed for the diagnosis of AH had 58% mortality related to biopsy [43]. However, larger studies reported later have shown that a trans jugular liver biopsy had acceptable success with a low morbidity or mortality [44]. In a systematic review of 64 studies on 7649 subjects with any liver disease and undergoing trans jugular liver biopsy, minor and major complications were reported in 6.5% and 0.6% respectively. With a mortality rate of 0.09% in this pooled analysis, the outcomes were superior at centers with experience of performing >100 trans jugular biopsies. With a median 3 passes through the liver, the success rate in obtaining an adequate tissue was 97%, which could provide a definite histological diagnosis in 96% cases.[44] It should be recognized that the trans jugular biopsy may only be available in specialized tertiary centers, as the technique requires expertise and infrastructure. Endoscopic ultrasound (EUS) guided liver biopsy is being increasingly used for liver biopsy, and allows for sampling of the right and left liver lobes.[45] While EUS-guided liver biopsy has a diagnostic yield comparable to the trans jugular approach[46 47], these studies are limited with their retrospective design and inclusion of only 0-15% of patients with AH and/or cirrhosis. Clinical trials comparing the diagnostic yield, tissue quality, and safety of EUS-guided liver biopsy in AH patients is currently underway (Clinical trial identifier NCT04235855).

When a liver biopsy is performed, the pathologist should examine for specific findings of AH such as macro vesicular steatosis, lobular neutrophils, hepatocyte injury with ballooning degeneration and/or Mallory-Denk bodies, bilirubinostasis and fibrosis in a pericellular or sinusoidal pattern(i.e. chicken wire fibrosis) (Figure 2).[5, 17, 42, 48] Identification of underlying cirrhosis is important as its presence in AH patients negatively impacts the patient survival. However, there is inter-observer variability of 42-100% in detection and staging of fibrosis on liver biopsy, including 15-20% sampling variation on the identification of cirrhosis on a liver biopsy [49-51]. It is reported that the majority of AH patients have underlying cirrhosis, although the prevalence rates have varied from 30-100% in different series.[5, 6, 52]

The literature is conflicting regarding the role of liver biopsy for diagnosis of AH [37, 40, 43, 52-55]. One of the reasons for variability on diagnosis of AH using liver biopsy is an inter-observer variability between pathologists on specific findings of AH. For instance, bilirubinostasis has intra-observer agreement ranging from 52-86% [49-51]. Similarly, inter-observer variability has been reported for mega mitochondria (20%-46%) [49-51] and hepatic steatosis (43-89%) [40, 51]. Of the various histological findings of AH, the agreement between pathologists is excellent for cholestasis, modest for neutrophilic lobular infiltration, and poor for mega mitochondria, with kappa statistics of 0.86, 0.6, and 0.46 respectively.[49] In another study, the kappa statistics for agreement between pathologists on interpretation of liver biopsy for histological findings of AH was 0.89 for steatosis, 0.60 for portal fibrosis, 0.65 for lobular inflammation, , and 0.40 for hepatocyte ballooning.[40] A recent study by the Study of Alcohol-related LiVer disease in Europe (SALVE) Histopathology Group on a multicenter cohort of 445 patients with ALD aimed at developing and validating a scoring system (SALVE grade) for ALD showed kappa statistics of 0.88 for steatosis and 0.66 for neutrophilic lobular infiltration.[56] The study showed that histological features of AH (hepatocyte ballooning and neutrophilic lobular infiltration) and severe cirrhosis (fibrosis assessment based on NASH CRN and Laennec's staging systems) predicted survival and decompensation events in the short-term.[56]

The histological findings should be interpreted in light of the clinical data, as histological findings of AH have been reported without the clinical phenotype of AH, [7] and have been known to be present in explants of liver transplant recipients for alcohol associated cirrhosis after abstaining from alcohol for ≥6 months.[57, 58] For accurate interpretation on histological findings of AH, at least five portal triads should be present, and the histology be independently interpreted by at least two pathologists with expertise in liver pathology. As the histological findings may change quickly after treatment with corticosteroids,[59] the liver tissue should ideally be examined before administration of corticosteroids. It may often be needed to send out the frozen sections to specialized centers with expertise in reading the liver tissue for

findings of AH. This is often feasible as the histological findings of AH can persist for several months after alcohol cessation, thus providing a wide diagnostic window.[36, 37, 57, 58]

The prognostic role of liver biopsy in AH and ACLF

Histological findings of AH have been used to estimate disease prognosis and response to medical treatment. In one study on AH patients recruited from multiple centers, the alcoholic hepatitis histologic score (AHHS) had a receiver operating characteristic value of 0.77 in predicting 90-day mortality, with mortality rates of 3%, 19%, and 51% at AHHS 0-3, 4-5, and 6-9 respectively.[49] AHHS includes bilirubinostasis, fibrosis (presence and degree correlates with more severe disease), mega mitochondria and neutrophilic infiltration (presence and degree correlates with less severe disease). Although, increased serum white-blood cell count confers higher risk of mortality and acute kidney injury in patients with AH [6, 60, 61]neutrophilic infiltration within the liver lobules appears to have mixed effects. On one hand, neutrophils are thought to cause persistent oxidative stress [62] and hepatic inflammation due to the formation of neutrophil extracellular traps [63] however they may also be hepato-protective due to their ability to clear necrotic hepatocytes and promote hepatic regeneration secondary to release of hepatocyte growth factor[64].Whileutility of AHHS in predicting mortality has been replicated in few other studies, [59, 65] the AHHS was not superior to the clinical non-invasive assessment including MELD and modified discriminant function (DF) scores.[49, 59] For individuals with moderate AH (MELD <21), the AHHS cut-off value of 5 has utility to discriminate 72% and 94% survival rates at 90 days.[49]Further work is needed to determine if liver biopsy findings have better prediction of disease severity and patient survival among patients with moderate AH (MELD 11-20) or DF <32.

Of the individual components of AH, bilirubinostasis has been shown to predict bacterial infections, sepsis, and mortality [49, 66] Role of histology in predicting response to steroids remains controversial, with ballooning degeneration and Mallory-Denk bodies being associated with non-response to corticosteroids in one study, [67] and lack of correlation of the

AHHS in another study.[49] More studies are needed to determine how liver biopsy can be utilized to guide treatment and inform prognosis in AH and ACLF.

Non-invasive modalities

Given the limitations of liver biopsy as described above, liquid liver biopsy with non-invasive biomarkers has emerged for the diagnosis of AH and predicting its prognosis [68, 69] For example, levels of circulating extracellular vesicles have been shown to be three fold elevated among patients with AH compared to healthy individuals [70]. In another study on AH patients, elevated levels of circulating exosomes and sphingolipid cargo within these vesicles improved the accuracy of MELD score in predicting patient survival. [71]Cytokeratin-18 released as a result of hepatocyte injury and its cleaved and uncleaved forms (*M65 and M30*) have also shown a potential for diagnosis of AH, predicting prognosis, and response to medical treatment [72].

Over the last few years, several other biomarkers have been derived for non-invasive diagnosis of AH and predicting prognosis in these patients. These biomarkers have been derived from: a) ***serum:*** malondialdehyde [73], osteopontin[74], CCL-20 [69, 75] and IL-6 [76]; b) ***stool***: changes in the gut microbiota[77]; c) **breath**: trimethylamine, pentane, 2-propranolol, acetone, acetaldehyde, and ethanol; [78] d) ***peripheral blood mononuclear cells***: mitochondrial function and oxygen consumption rate; [69, 79] and e) ***isolated DNA:*** genetic polymorphisms of patatin-like phospholipase domain protein 3 (*PNPLA*3), *TM6SF2, MBOAT7, and HSD17B13*.[80-83]

Conclusions and Future Directions

Among patients with decompensated ALD and ACLF, there should be low threshold for suspecting AH. Although, clinical diagnosis of AH has improved using the criteria proposed by the NIAAA, a liver biopsy may be needed among patients with uncertain clinical diagnosis. A trans jugular route is preferred in these sick patients with ascites and/or coagulopathy. While

data are conflicting on the role of liver biopsy in predicting disease severity and patient survival, several non-invasive biomarkers are emerging. Lack of validation, technical difficulties, lack of widespread availability, and high cost, however, limit their current use in routine clinical practice.[69, 83]

In conclusion, liver biopsy should be considered on a case-by-case basis for ALD patients presenting with ACLF and a possible or probable diagnosis of AH. Clearly, the data gathered from ongoing clinical trials will be useful to further substantiate the role of liver biopsy for diagnosis of AH and predicting its prognosis. The clinical trajectories of patients with biopsy-proven, definite AH in the setting of ACLF should define the role of liver biopsy in guiding therapy and prognosis of patients with ALD and ACLF.

Table 1 Criteria to define acute on chronic liver failure (ACLF) by various consortia

Variable	AARC	EASL-CLIF	NACSELD
Underlying liver disease	CLD or cirrhosis	Cirrhosis	Cirrhosis
Precipitating event/s	Any	Any	Only infection
Liver failure	Bilirubin Ascites	Bilirubin	Not included
Renal failure	Creatinine or RRT	Creatinine or RRT	Only RRT
Coagulation failure	INR	INR	Not included
Brain failure	HE grade 3 or 4	HE grade 3 or 4	HE grade 3 or 4
Respiratory failure	Not included	PaO2/FiO2 <214 Mechanical ventilation	Mechanical ventilation
Circulatory failure	Not included	Vasopressor/s	MAP<60 or decrease in SBP by >40 mm Hg

AARC: Asia-Pacific ACLF Research Consortium; CLIF: Chronic Liver Failure Consortium; NACSELD: North American Consortium for End-stage Liver Disease; CLD: Chronic Liver Disease; RRT: Renal Replacement Therapy; INR: International Normalized Ratio; HE: Hepatic Encephalopathy; MAP: Mean Arterial Pressure; SBP: Systolic Blood Pressure

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Legends to Figures

Figure 1 Clinical criteria and role of liver biopsy for the diagnosis of alcoholic hepatitis (AH) among patients with alcohol-associated liver disease and acute on chronic liver failure (ACLF).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, Gamma-glutamyl transferase.

Figure 2 Liver biopsy findings of severe alcoholic hepatitis.
Macrovesicular steatosis with Mallory hyaline (panel 1), intracanalicular and ductular cholestasis (panel 2), and neutrophilic infiltration of lobules and hepatocytes (panel 3), and advanced bridging fibrosis to evolving cirrhosis (panel 4).
(Adapted from: Axley et al. J Clin Transl Hepatol 2017; 5: 1-2).

Table 1 Criteria to define acute on chronic liver failure (ACLF) by various consortia

Variable	AARC	EASL-CLIF	NACSELD
Underlying liver disease	CLD or cirrhosis	Cirrhosis	Cirrhosis
Precipitating event/s	Any	Any	Only infection
Liver failure	Bilirubin Ascites	Bilirubin	Not included
Renal failure	Creatinine or RRT	Creatinine or RRT	Only RRT
Coagulation failure	INR	INR	Not included
Brain failure	HE grade 3 or 4	HE grade 3 or 4	HE grade 3 or 4
Respiratory failure	Not included	PaO2/FiO2 <214 Mechanical ventilation	Mechanical ventilation
Circulatory failure	Not included	Vasopressor/s	MAP<60 or decrease in SBP by >40 mm Hg

AARC: Asia-Pacific ACLF Research Consortium; CLIF: Chronic Liver Failure Consortium; NACSELD: North American Consortium for End-stage Liver Disease; CLD: Chronic Liver Disease; RRT: Renal Replacement Therapy; INR: International Normalized Ratio; HE: Hepatic Encephalopathy; MAP: Mean Arterial Pressure; SBP: Systolic Blood Pressure