

Histologic Changes in Core-Needle Liver Biopsies From Patients With Acute-on-Chronic Liver Failure and Independent Histologic Predictors of 28-Day Mortality

Vandana Baloda, MD; Abhinav Anand, DM; Rajni Yadav, MD; Lalita Mehra, MSc; Madhu Rajeshwari, MD; Sreenivas Vishnubhatla, PhD; Ashish Datt Upadhyay, PhD; Sada N. Dwivedi, PhD; Baibaswata Nayak, PhD; Anoop Saraya, DM; Subrat K. Acharya, DM; Siddhartha Datta Gupta, MD; Shalimar, MD; Prasenjit Das, MD, DNB

• **Context.**—The histologic features in patients with acute-on-chronic liver failure (ACLF) are evolving, and histologic indicators of patients' poor prognosis are not yet fully established.

Objective.—To evaluate the independent histologic predictors of 28-day mortality in ACLF patients on core-needle liver biopsies.

Design.—Core-needle biopsies from patients with a diagnosis of ACLF (n = 152) as per the European Association for the Study of the Liver criteria were included during 8 years. Liver biopsies from 98 patients with compensated chronic liver disease were included as disease controls for histologic comparison. Features of ongoing changes, such as hepatic necrosis, hepatic apoptosis, cholestasis, hepatocyte degeneration, bile ductular proliferation, Mallory-Denk bodies, steatosis, and extent of liver fibrosis, were analyzed for predicting short-term mortality (28 days). A *P* value of <.05 was considered significant.

Results.—In our cohort of ACLF patients, the following etiologies for acute decompensation were identified: alcohol, 47 of 152 (30.9%); sepsis, 24 of 152 (15.7%); hepatotropic viruses, 20 of 152 (13.1%); drug-induced

liver injury, 11 of 152 (7.2%); autoimmune flare, 9 of 152 (5.9%); mixed etiologies, 5 of 152 (3.2%); and cryptogenic, 36 of 152 (23.6%). On histologic examination, hepatic necrosis (*P* < .001), dense lobular inflammation (*P* = .03), cholestasis (*P* < .001), ductular reaction (*P* = .001), hepatocyte degeneration (*P* < .001), and absence of advanced fibrosis stages (*P* < .001) were identified significantly more often in ACLF patients than in disease controls on univariate analysis. On multivariate Cox regression analysis, the absence of advanced Ishak histologic activity index fibrosis stages (*P* = .02) and the presence of dense lobular inflammation (*P* = .04) were associated with increased 28-day mortality in ACLF patients. After adjusting the clinical causes of acute decompensation, only dense lobular inflammation was found as an independent predictor of short-term mortality (*P* = .04) in ACLF patients.

Conclusions.—Dense lobular necroinflammatory activity is a clinically independent histologic predictor of 28-day short-term mortality in patients with ACLF.

(*Arch Pathol Lab Med.* 2022;146:846–854; doi: 10.5858/arpa.2021-0103-OA)

Acute-on-chronic liver failure (ACLF) syndrome is characterized by abrupt hepatic decompensation secondary to acute insult in patients with chronic liver disease (CLD), having high short-term mortality.^{1–3} The diagnosis and outcomes are defined by clinical criteria and prognostic scores, considering the extent of organ failures.^{4,5} Liver histology can be helpful in situations when the etiology of

acute decompensation is unclear and to differentiate between acute liver failure and ACLF in patients with previously undocumented CLD. Establishing the nature and extent of acute hepatic insult in a patient with CLD is also an indication.⁴ However, liver biopsy has its inherent risks of being an invasive procedure. Only a few studies have analyzed if the histologic features of acute hepatic decompensation, such as hepatocyte necrosis, hepatocyte degeneration, the density of lobular inflammation, bile ductular proliferation, cholestasis, fibrosis, etc, can be prognostically important. Histologic features in patients with ACLF were also classified into 2 pattern groups with distinct clinical outcomes.^{5–8} Establishing independent histologic prognostic features will impact clinical decision-making as a direct marker of hepatocyte reserve.^{9–13} In a study based on a prospectively maintained ACLF case database, we analyzed the core-liver biopsies taken from patients with ACLF and semiquantitatively graded the severity of histologic indicators of acute hepatic derangement, and we compared them with the histologic features in compensated CLD patients.

Accepted for publication July 6, 2021.

Published online October 27, 2021.

Supplemental digital content is available for this article at <https://meridian.allenpress.com/aplm> in the July 2022 table of contents.

From the Departments of Pathology (Baloda, Yadav, Mehra, Rajeshwari, Gupta, Das), Gastroenterology (Anand, Nayak, Saraya, Acharya, Shalimar), and Biostatistics (Sreenivas, Upadhyay, Dwivedi), All India Institute of Medical Sciences, New Delhi, India.

The authors have no relevant financial interest in the products or companies described in this article.

Corresponding author: Prasenjit Das, MD, DNB, Department of Pathology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India (email: prasenaiims@gmail.com).

Histologic features were correlated with 28-day short-term mortality in this cohort.

MATERIALS AND METHODS

Patients With ACLF

In this retrospective cross-section study, we included core-needle biopsies from 152 patients with ACLF, fulfilling the European Association for the Study of the Liver consensus criteria, during the period of 2011 to 2018.⁴ Written informed consent was obtained from patients or the nearest attending relatives as per institutional protocol while taking the biopsies. Being a retrospective histomorphologic study, separate ethical clearance was not applicable. As per the European Association for the Study of the Liver criteria, ACLF was defined as an acute decompensation of CLD that was associated with organ failures and high short-term mortality.⁴ The clinical details, including blood investigations, etiologic workup, and mortality data within 28 days of hospitalization, were obtained from a prospectively maintained database.

Controls With Disease

Liver core biopsies from 98 patients with compensated CLD were included as disease controls randomly from our database. This group of patients had nonalcoholic fatty liver disease–related cirrhosis (50 [51.02%]), autoimmune hepatitis (25 [25.51%]), viral hepatitis (17 [17.34%]), and cryptogenic cirrhosis (6 [6.12%]). The controls were neither matched for the etiology of cirrhosis nor stage-matched with the ACLF group. The formalin-fixed, paraffin-embedded blocks were retrieved from the archive to compare the histologic findings in ACLF versus compensated CLD patients.

Liver Biopsies

Transjugular liver biopsies were taken with an 18-G Cook needle from 25 ACLF patients who survived the initial insult for at least 24 hours to look for pathologic changes in the liver, and in the rest (n = 127), transcutaneous postmortem core-needle biopsies were performed within 30 minutes of death for establishing cause and etiology of liver failure. In all compensated CLD patients, transcutaneous liver core biopsies were taken for establishing etiologic diagnoses and fibrosis staging. The formalin-fixed, paraffin-embedded blocks, and slides stained with hematoxylin-eosin, silver reticulin, Masson trichrome, and periodic acid-Schiff were retrieved from records. Sections were cut, and orcein stain was performed to differentiate between bridging fibrosis and bridging necrosis wherever needed. Two experienced pathologists reviewed the slides individually in a blinded manner, and the findings were later correlated and finalized. Criteria for inclusion of the liver biopsies were the presence of at least 6 portal tracts and composite core length of at least 1.5 cm. Exclusion criteria were inadequate biopsy size or portal tract numbers and autolyzed tissue.

Histomorphologic Changes

The necroinflammatory activity in the liver biopsies from patients with ACLF and controls with disease was graded as per the Ishak histologic activity index (HAI) grading system.¹⁴ For statistical analysis, necroinflammatory scores 3 and 4 were considered as severe for portal inflammation, interface hepatitis, and lobular inflammation. Lobular inflammation was defined by lymphocytic spotty necrosis, neutrophilic infiltrate, and infiltration by ceroid-laden macrophages. We incorporated both the Ishak staging system and the French METAVIR Cooperative Study Group staging system of liver fibrosis for hepatic fibrosis assessment.^{14,15} METAVIR stages 3 and 4 were considered as advanced fibrosis. Histologic features, such as hepatocyte ballooning, Mallory-Denk bodies (MDBs), hepatocyte regeneration, apoptosis, cholestasis, feathery degeneration, and bile ductular proliferation, were semiquantitatively graded following a 3-scale semiquantitative grading system for the convenience of histologic analyses as 0, none; 1, mild (focal/patchy); and 2, moderate to marked (diffuse). The ballooned hepatocytes were defined as swelling of hepatocytes with rounding of cytoplasmic borders and

having vacuolated, rarefied, and reticulated cytoplasm. Regenerative hepatocyte was defined by the presence of nuclear pleomorphism and multinucleation in a mature hepatocyte. Apoptotic bodies were defined as small dysmorphic hepatocytes having dense eosinophilic cytoplasm with pyknotic and karyorrhectic nuclei. Canalicular, cytoplasmic, and ductular cholestasis were identified and taken as features of acute liver injury, along with hepatocyte necroinflammatory activity, ballooning, feathery degeneration, presence of acidophil bodies, MDBs, ductular proliferation, and hepatocyte necrosis. Dilated biliary ductules with intraluminal bile plug and neutrophils were taken as sepsis-related changes. Feathery degeneration was defined as swelling of hepatocytes with cytoplasmic rarefaction and the presence of cytoplasmic bile pigments. The presence of large punched-out cytoplasmic vacuole/s indenting and pushing the nuclei to the periphery was taken as macrovesicular steatosis. The presence of macrovesicular steatosis in >5% liver core was considered to be of pathologic significance, and these liver biopsies were graded and staged as per the Nonalcoholic Steatohepatitis Clinical Research Network grading and staging system.¹⁶ The presence of microvesicular steatosis was defined by foamy hepatocytes with fine cytoplasmic fat vacuoles not indenting the nuclei with a high surface-to-volume ratio. Foci of confluent and/or bridging necrosis were identified on hematoxylin-eosin–stained sections and confirmed by orcein stain where needed.¹⁷ Patterns of fibrosis, in the form of portal, periportal, bridging, or pericellular fibrosis, were analyzed.¹⁷ Periportal fibrosis was defined by the presence of coarse parallel reticulin fibers in the periportal region. Coagulative necrosis was graded as 0, absent; 1, occupying less than 50% of the core area; and 2, occupying more than 50% of the core area. This simplified in-house designed grading system was followed instead of Ishak 6-tier anatomic grading of the confluent necrosis to reduce the overall subgrades and for the sake of histologic comparison. We included liver biopsies from patients with compensated CLD for histologic comparison because histologic features in decompensated cirrhosis will mimic the changes in ACLF patients. The histologic features were also classified as pattern I change, including bile ductular proliferation (grades 2 and 3), frequent acidophil bodies, confluent/bridging necrosis (grades 2 and 3), high apoptosis (grades 2 and 3), pericellular fibrosis, MDBs (grades 2 and 3), coarse canalicular and ductular cholestasis, and advanced fibrosis (analyses were performed separately with combined stages 5 and 6, as well as fibrosis stages ≥ 3); and pattern II changes, including hepatocyte ballooning (grades 1 and 2), cholestasis (mild granular intracytoplasmic), mild focal apoptosis/necrosis (grade 1), and lower stages of fibrosis (HAI ≤ 3), as described by Rastogi et al,⁸ and were correlated with patient survival data.

Statistical Analysis

Data were presented as mean (\pm SD), median (interquartile range [IQR]), or frequency (percentage). For comparison of categorical variables, we applied the χ^2 test or Fisher exact test as appropriate. For continuous variables following normality, we applied an independent *t* test. For continuous variables not following a normal distribution, we applied the Wilcoxon rank-sum test. Kaplan-Meier survival analysis was done to assess the survival with each exposure variable, followed by a log-rank test for assessment of the significance of the distribution. Multivariate stepwise Cox regression analysis was carried out to quantify the hazard of short-term mortality associated with the histologic features that were statistically significant in univariate analysis. A *P* value of <.05 was taken as significant. Statistical analysis was performed with Stata 15.1 software (Stata Corp LLC, College Station, Texas).

RESULTS

Demographic, Clinical, and Biochemical Profiles

The median (IQR) age of the patients with ACLF was 40 years (range, 30–50 years), and 115 (75.65%) were male. All clinical features were not uniformly documented in the 152 ACLF patients included, hence their denominators differ in

Table 1. Baseline Laboratory Characteristics of Patients With Acute-on-Chronic Liver Failure (ACLF) and Compensated Chronic Liver Disease (CLD; Controls With Disease)^a

Variable ^b	ACLF (n = 152)	Compensated CLD (n = 98)	P Value
Age, y	40 (30–50)	40 (29–50)	.84
Hemoglobin, g/dL	9.4 ± 2.4	12.1 ± 2.6	<.001
TLC per mm ³	12 900 (8950–20 150)	7300 (4745–8900)	<.001
Platelets, × 10 ³ /μL	105 (70–190)	171 (91–228)	.003
Bilirubin, mg/dL	17.0 (7.3–24.7)	0.8 (0.5–1.4)	<.001
AST, IU/L	137.0 (78.8–231.3)	49.0 (31.0–75.5)	<.001
ALT, IU/L	72.0 (42.0–151.3)	51.0 (32.0–92.0)	.001
ALP, IU/L	237.0 (170.0–337.0)	273.0 (204.8–430.5)	.01
INR	2.5 (1.9–3.6)	1.3 (1.0–1.5)	<.001
Albumin, g/dL	2.5 (2.1–3.0)	4.2 (3.5–4.8)	<.001
Urea, mg/dL	63.5 (32.3–110.8)	23.0 (18.0–29.0)	<.001
Creatinine, mg/dL	1.6 (0.8–3.5)	0.8 (0.5–1.4)	<.001

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; TLC, total leukocyte count.

^a Values in parentheses indicate range of readings for that particular parameter.

^b The conversion factors to SI units are as follows: bilirubin, ×17.104; albumin, ×10; urea, ×0.357; creatinine, ×88.4; AST, ×0.0167; ALT, ×0.0167; and ALP, ×0.0167.

Table 1. Hepatic encephalopathy and gastrointestinal bleeds were documented in 109 of 141 (77.3%) and 46 of 135 (34.1%) ACLF patients, respectively. Hematologic and laboratory parameters are shown in Table 1. The ACLF patients in comparison with controls had low hemoglobin ($P < .001$), and higher total leukocyte count ($P < .001$), bilirubin ($P < .001$), creatinine ($P < .001$), urea ($P < .001$), aspartate aminotransferase ($P < .001$), alanine aminotransferase ($P = .001$), and alkaline phosphatase ($P = .02$) levels. The mean ± SD Child-Turcotte-Pugh score was 11.86 ± 1.67 . The median (IQR) Model for End-Stage Liver Disease score was 32.2 (27.2–40.0), and the acute physiology and chronic health evaluation score was 17 (10.0–22.0) in our cohort of ACLF patients.

Causes of Acute Decompensation in Our Cohort of ACLF Patients

The clinically identified causes of acute decompensation in our cohort of ACLF patients were as follows: alcohol (47 of 152; 30.9%), followed by sepsis (24 of 152; 15.7%) and hepatotropic viral infection (20 of 152; 13.1%). Additionally, antitubercular therapy and autoimmune flares were implicated in 11 of 152 (7.2%) and 9 of 152 (5.9%) cases, respectively. More than 1 acute etiologic event was seen in 5 of 152 patients (3.2%). In the remaining 36 of 152 (23.6%), the etiology of acute decompensation could not be ascertained. Overall, 127 of the 152 ACLF patients whose liver biopsies were included in this study cohort died within 28 days of hospitalization (83.6% short-term mortality). None of the causes of acute decompensation was found significant for short-term mortality in univariate analysis, so multivariate analysis was not done (alcohol, assumed as reference: odds ratio [OR] = 1); viral hepatitis: OR, 0.773 (95% CI, 0.173–3.445), $P = .74$; sepsis: OR, 0.784 (95% CI, 0.201–3.061), $P = .73$; antitubercular therapy: OR, 0.239 (95% CI, 0.053–1.065), $P = .06$; and autoimmune hepatitis: OR, 0.273 (95% CI, 0.054–1.388), $P = .12$.

Histomorphologic Changes

Liver Biopsies From ACLF Patients.—Detailed histologic changes are described in Table 2. A total of 57 of 152

liver biopsies (37.5%) from ACLF patients showed the presence of confluent necrosis, out of which confluent necrosis involving >50% of core area was noted in 30 liver biopsies (19.7%; Figure 1, A through D). Bridging necrosis was present in 15 cases (9.8%; Figure 1, C). Cholestasis was identified in 144 of 148 biopsies (97.3%), including combined hepatocellular and canalicular cholestasis in 53 (35.8%) and ductular cholestasis in 62 (41.9%) liver biopsies (Figure 1, C through F). Macrovesicular steatosis was noted in 89 of 149 (59.7%; Figure 2, A), and microvesicular steatosis was seen in 30 of 149 (20.1%; Figure 2, B) liver biopsies. The common acute etiologies associated with histologic evidence of microvesicular steatosis were alcohol (10 of 30; 33.33%), sepsis (8 of 30; 26.66%), antitubercular therapy (3 of 30; 10.00%), and idiopathic acute event (4 of 30; 13.33%), along with acute hepatitis E virus infection, acute hepatitis A virus infection, viral reactivation, acute autoimmune event, and variceal bleed in 1 of 30 cases (3.33%) each. The most common chronic etiologies associated with microvesicular steatosis were alcohol (12 of 30; 40%), chronic viral hepatitis (7 of 30; 23.33%), cryptogenic chronic etiology (6 of 30; 20%), Wilson disease (1 of 30; 3.33%), nonalcoholic fatty liver disease (1 of 30; 3.33%), hepatic venous outflow tract obstruction (1 of 30; 3.33%), and autoimmune hepatitis (2 of 30; 6.66%). Some of the histologic features could not be analyzed in all 152 liver biopsies for various technical reasons because there were no viable leftover hepatocytes, or the stains were faded, being a retrospective study, or there was nonagreement between the pathologists due to loss of nuclear stain in a minor subset of biopsies. MDBs were identified in 69 of 148 liver biopsies (50.67%) from ACLF patients (Figure 2, C and D). Dense portal tract inflammation involving most of the portal tracts was noted in 28 biopsies (18.5%), predominantly comprising lymphocytes (Figures 1, A and B, and 2, E and F). Diffuse interface hepatitis involving all portal tracts and prominent ductular reactions were noted in 110 (73.8%) and 90 (60%) of these biopsies, respectively (Figure 2, B, E, and F). Lobular inflammatory cell infiltrate was noted in all these liver biopsies (Figure 1, A and B). Severe lobular inflammation, including prominent ceroid-laden macrophages, was identified in 21 of 148 biopsies (14.2%; Figure 1, C through E).

Table 2. Comparison of Histologic Features Noted in Liver Biopsies From Patients With Acute-on-Chronic Liver Failure (ACLF) and Compensated Chronic Liver Disease (CLD)

Histologic Parameters	ACLF (n = 152), No. (%)	Compensated CLD (n = 98), No. (%)	P Value ^a
Hepatic necrosis	57/152 (37.50)	0/98 (0)	<.001
Apoptotic bodies	137/150 (91.35)	41/98 (41.8)	<.001
High apoptotic bodies	77/150 (51.33)	3/98 (3.06)	<.001
Macrovesicular steatosis	72/149 (48.32)	61/98 (62.24)	.004
Moderate to diffuse macrovesicular steatosis	30/149 (20.13)	25/98 (25.51)	.33
Microvesicular steatosis	30/149 (20.13)	2/98 (2.04)	<.001
Cholestasis	144/148 (97.30)	39/98 (39.8)	<.001
Significant cholestasis	98/148 (66.21)	8/98 (8.16)	<.001
Severe lobular inflammation including ceroid-laden macrophages	21/148 (14.19)	5/98 (5.02)	.02
Dense portal inflammation	28/151 (18.54)	8/98 (8.16)	.02
Interface hepatitis (diffuse, involving most portal tracts)	110/149 (73.83)	59/98 (60.2)	.02
Prominent ductular reaction	90/150 (60)	23/98 (23.47)	<.001
Multinucleation	144/149 (96.64)	83/98 (84.69)	.001
Prominent multinucleation	69/149 (46.30)	19/98 (19.38)	<.001
Ballooned hepatocytes	144/148 (97.3)	94/98 (95.92)	.55
Diffuse hepatocyte ballooning	67/148 (45.25)	12/98 (12.24)	<.001
Feathery degeneration	75/148 (50.68)	4/98 (4.12)	<.001
Mallory-Denk bodies	69/148 (50.67)	25/98 (25.51)	<.001
Numerous Mallory-Denk bodies	36/148 (24.32)	3/98 (3.06)	<.001
Ishak HAI stages 5 and 6 (advanced)	127/150 (84.67)	48/98 (48.98)	<.001
METAVIR stages 3–4 (advanced)	131/148 (88.51)	54/98 (55.1)	<.001

Abbreviation: HAI, histologic activity index.

^a Bold entries refer to statistically significant *P* values.

Hepatocyte ballooning and feathery degeneration were noted in 144 (97.3%) and 75 (50.7%) biopsies, respectively (Figure 1, C, D, and F). Apoptotic bodies, on the other hand, were identified in 137 of 150 liver biopsies (91.4%; Figure 2, E). The HAI stages 5 to 6 (advanced fibrosis) were present in 127 (84.7%) and METAVIR stages 3 to 4 (advanced fibrosis) were present in 131 (88.5%) of these biopsies (Figures 1, A, and 2, E and F; Table 2). Only 23 of our ACLF patients had Ishak fibrosis stages 1 to 4, and 17 liver biopsies had METAVIR fibrosis stages 1 to 2 (Table 2 and Supplemental Table 1 at <https://meridian.allenpress.com/aplm> in the July 2022 table of contents). The pattern I histologic change was seen in 141 of 152 ACLF patients (92.76%), and pattern II histology was seen in 80 of 152 ACLF patients (52.63%) in our cohort with overlap.

Liver Biopsies From Disease Controls.—The histopathologic features were consistent with the clinical diagnoses mentioned and have been detailed in Table 2. In these liver biopsies, the HAI stages 5 to 6 were present in 48 of 98 (48.9%) and METAVIR stages 3 to 4 were present in 54 (55.1%) of these biopsies (Table 2). None of these liver biopsies had features of confluent and bridging necrosis. Cholestasis was identified in 39 of 98 (39.8%) of them (Table 2).

Comparison of Histologic Features Between ACLF and Compensated CLD

Liver biopsies from patients with ACLF in comparison with compensated CLD biopsies showed significantly more confluent necrosis ($P < .001$), moderate to numerous apoptotic bodies ($P < .001$), moderate to severe cholestasis ($P < .001$), microvesicular steatosis ($P < .001$), lobular inflammation ($P = .03$), portal inflammation ($P = .03$), diffuse interface hepatitis involving all portal tracts ($P = .02$),

ductular proliferation ($P < .001$), and ductular dilatation/cholestasis ($P < .001$). Other degenerative changes in the hepatocytes, such as severe ballooning ($P < .001$), feathery degeneration ($P < .001$), MDBs ($P < .001$; Figure 2, C and D), and advanced hepatic fibrosis (Ishak stages 5–6 and METAVIR stages 3–4) were also significantly greater in liver biopsies from ACLF patients than in compensated CLD controls (Table 2). In the former group, hepatocyte regeneration ($P < .001$) was also significantly prominent (Table 2).

Histopathologic Parameters as Predictors of Outcome in ACLF Patients

Although most of the histologic features of acute necroinflammatory activity–mediated changes noted in our ACLF patients can be responsible for short-term mortality (Tables 2 and 3), hepatic necrosis (grades 2 and 3), dense lobular inflammation, and lack of advanced Ishak fibrosis stages (Ishak stages 5–6) were found as histologic predictors of poor prognosis on univariate analysis (Table 3). The median survival of ACLF patients with hepatocyte necrosis was 5 days (hazard ratio [HR], 1.64; 95% CI, 1.11–2.41), $P = .02$. The median survival in patients with moderate to marked lobular inflammation on liver biopsies was 4 days (HR, 1.73; 95% CI, 1.04–2.87), $P = .03$. The hazard of mortality in patients with advanced fibrosis (Ishak HAI 5 and 6) on liver biopsies was 8 days (HR, 0.52; 95% CI, 0.32–0.84), more than that where advanced fibrosis was not present, $P = .008$. On multivariate analysis, the absence of advanced Ishak fibrosis stages on liver core biopsies ($P = .02$) and dense lobular inflammation ($P = .04$) were found as histologic predictors of 28-day short-term patient mortality (Figure 3; Table 3). After adjusting the clinical causes of acute decompensation, only dense lobular inflammation

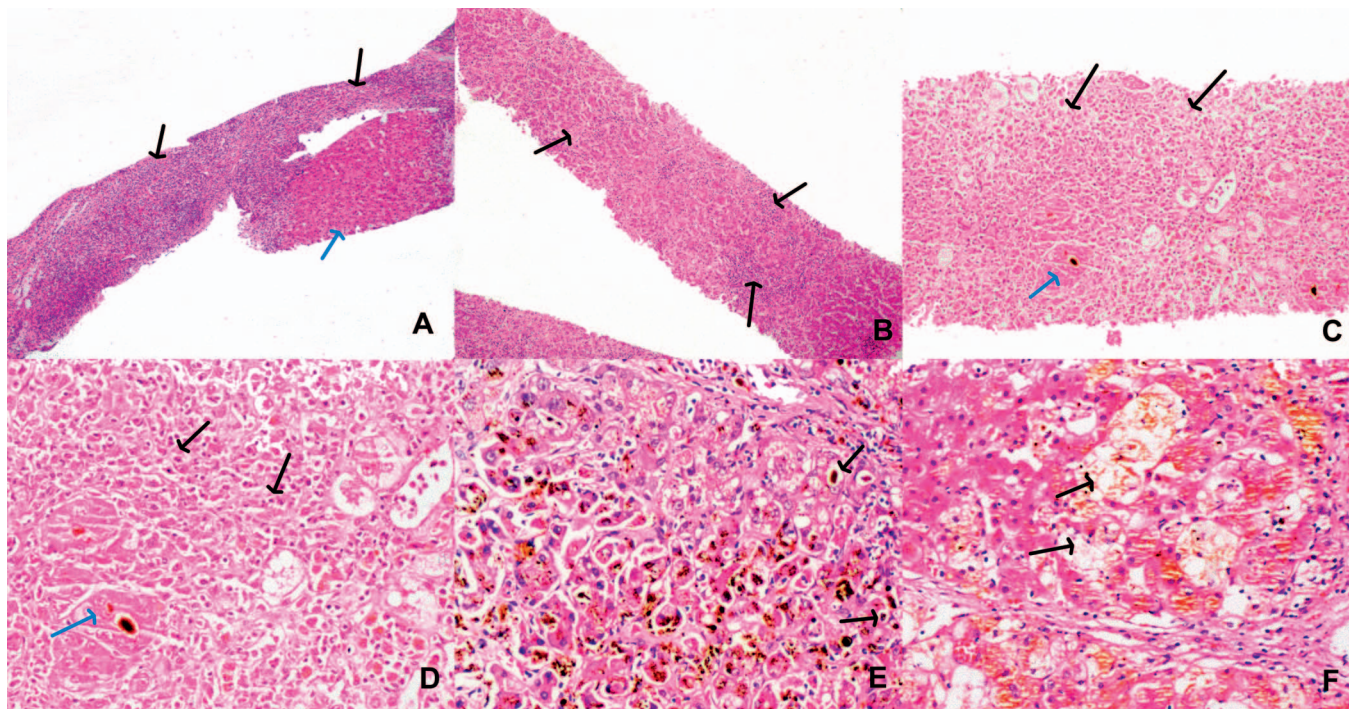


Figure 1. Low-power photomicrographs of transjugular liver biopsy (TJLB) in patients with acute-on-chronic liver failure show liver cores with thick fibrous bands densely infiltrated with inflammatory cells (black arrows) and a few residual hepatic nodules (blue arrow) (A). Another core biopsy shows maintained lobular architecture but dense lobular necroinflammatory activity (arrows) (B). A liver biopsy shows extensive confluent necrosis (black arrows) with infiltration by ceroid-laden macrophages and an occasional residual hepatocyte with cholestatic rosette (blue arrows) (C and D). One TJLB shows cirrhotic nodules with extensive intracanalicular (arrows) and intracytoplasmic cholestasis (E). Extensive intracytoplasmic cholestasis and feathery degeneration of hepatocytes are seen (black arrows) (F) (hematoxylin-eosin, original magnifications $\times 40$ [A and B], $\times 100$ [C], and $\times 200$ [D through F]).

was found as an independent predictor of short-term mortality ($P = .04$) in these patients. Also, the pattern I histology was identified in 24 of 25 patients with ACLF who survived (96%) and 117 of 127 ACLF patients who did not survive (92.12%). Pattern II histology was identified in 10 of 25 patients with ACLF who survived (40%) and 70 of 127 patients with ACLF who did not survive (55.11%). Both pattern I and pattern II histologies were correlated with the patient's survival, although no statistically significant difference was noted: $P = .99$ (pattern I) and $P = .17$ (pattern II). Liver core histologies in different clinical etiologic groups were also analyzed; however, having too many groups, we did not find a significant difference.

DISCUSSION

The present study attempts to document the histologic changes in needle-core biopsies from patients with ACLF having a divergent etiologic background, compared with the changes in compensated CLD, and find out the differential histologic features in ACLF patients having short-term (within 28 days of hospitalization) mortality, in comparison with those who survived. As compared with controls with disease, patients with ACLF had low hemoglobin levels and high serum creatinine, urea, bilirubin levels with abnormal liver function tests, deranged international normalized ratio, and low albumin levels. In the ACLF patients, hepatic necrosis, number of apoptotic/acidophil bodies in the liver, microvesicular steatosis, severe cholestasis, dense lobular and portal inflammations, diffuse interface hepatitis, ballooning, and feathery degeneration of hepatocytes, MDBs, and higher stages of liver fibrosis were more common than

in the compensated CLD patients (Table 2). The presence of dense lobular inflammatory activity was found as a clinically independent histologic predictor of 28-day short-term mortality in patients with ACLF on multivariate analysis (Table 3). In 35.8% of our cohort of liver biopsies from ACLF patients, evidence of CLD was identified on the histologic ground and was clinically undocumented.

We observed features of acute hepatic derangements, including hepatocyte damage, ballooning, feathery degeneration, apoptotic bodies, cholestasis, necrosis, and MDBs, significantly more in ACLF patients than in randomly selected compensated CLD patients (Table 2). Because our criteria of searching patients with CLD in our database were without features of acute decompensation, the differences in histologic features of acute hepatic decompensation were expected between these 2 groups. Nonetheless, this indicates that these histologic features are indeed associated with acute decompensation of the liver in these patients. However, these histologic features of acute derangement were not uniformly present in all 152 liver biopsies included. Hepatocyte necrosis was noted in 57 of 152 (37.50%), MDBs in 69 of 148 (50.6%), feathery degeneration in 75 of 148 (50.68%), cholestasis in 144 of 148 (97.30%), apoptotic bodies in 137 of 150 (91.35%), and ballooning in 144 of 148 (97.3%) of our liver biopsies from ACLF patients (Table 2). Interestingly, except for the hepatocyte necrosis, which was noted only in liver biopsies of ACLF patients, all other histologic features of hepatocyte derangements showed overlapping presence both in patients with ACLF and those with compensated CLD, although the severity of these changes was significantly greater in ACLF patients (Table 2).

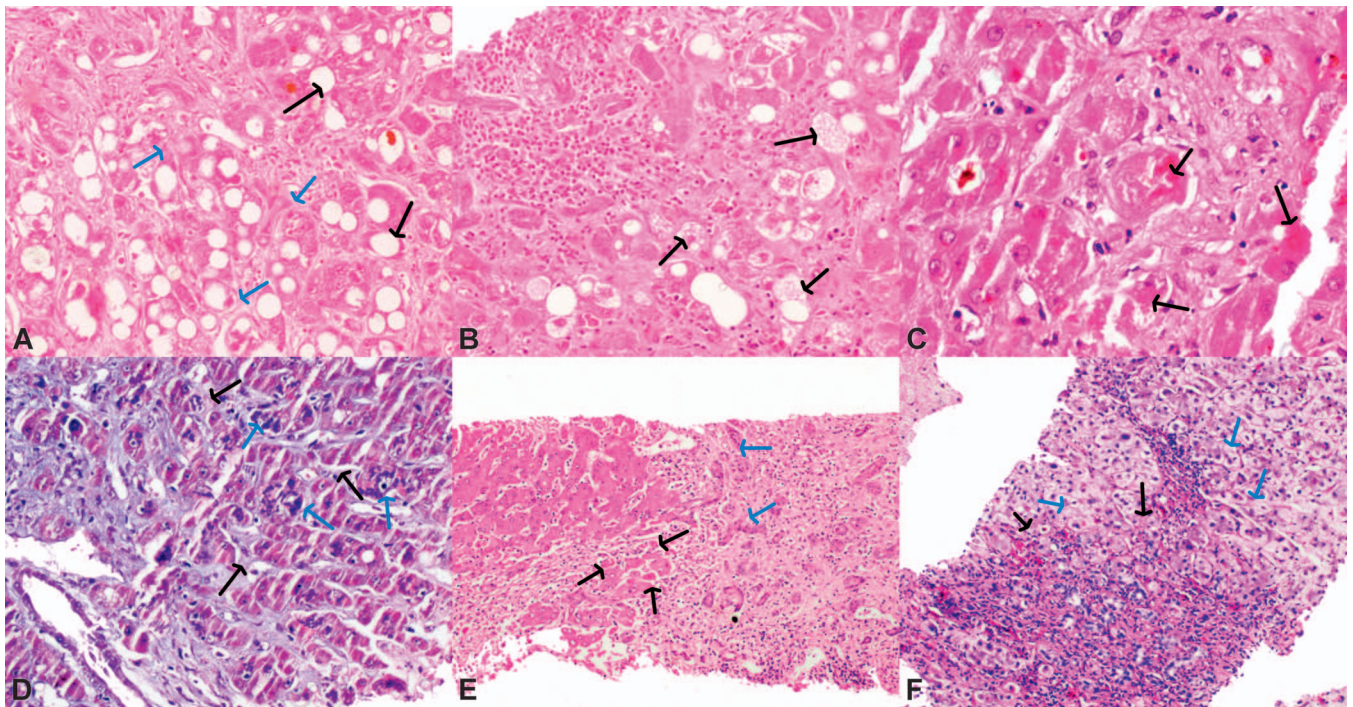


Figure 2. Liver core biopsy from a patient with acute-on-chronic liver disease shows diffuse macrovesicular steatosis (black arrows) with pericellular fibrosis (blue arrows) (A). Another liver biopsy from a chronic alcoholic patient shows diffuse microvesicular steatosis (arrows) with pericellular fibrosis and necroinflammatory activity (B). Prominent Mallory-Denk hyaline bodies (MDBs) are noted in hepatocytes (arrows) (C). Masson trichrome (MT) stain shows MDBs (blue arrows) and pericellular collagenization (black arrows) (D). A liver core biopsy shows septal inflammatory cell infiltrate, interface hepatitis, bile ductular proliferation (blue arrows), and prominent acidophil/apoptotic bodies (black arrows) (E). Along with dense portal inflammation, interface hepatitis (black arrows), and bile ductular proliferation, ballooned hepatocytes with acinar transformation (blue arrows) are noted (F) (hematoxylin-eosin, original magnifications $\times 200$ [A through C] and $\times 100$ [E]; MT, original magnification $\times 200$ [D]; and PAS-D, original magnification $\times 200$ [F]).

Especially, the severity of lobular inflammation and hepatocyte necrosis was found to be significantly greater in ACLF patients with documented short-term mortality (127 of 152 patients; 83.55%) than in ACLF survivors (Table 3). As per the inclusion criteria, liver biopsies from both ACLF and compensated CLD individuals had liver fibrosis; however, fibrosis stages were significantly higher in ACLF patients. It is again important to mention that in this study, CLD was defined by the presence of liver fibrosis of any extent, not limited to cirrhosis, and our controls were not stage-matched.

The spectrum of acute precipitants in our ACLF patients was like that in the Indian National Association for Study of the Liver consortium experience.¹⁸ This multicenter study described alcohol (35.7%), followed by hepatotropic viruses (21.4%), sepsis (16.6%), variceal bleed (8.4%), drugs (5.7%), and cryptogenic (9.9%), as the precipitating etiologies in Indian ACLF patients. In the index study, we also found alcohol (47 of 152; 30.9%), followed by sepsis (24 of 152; 15.7%), hepatotropic viruses (20 of 152; 13.1%), ATT (11 of 152; 7.2%), autoimmune flares (9 of 152; 5.9%), mixed etiologies (5 of 152; 3.2%), and cryptogenic (36 of 152; 23.6%), as causes of acute decompensation in ACLF patients. In the former study, multiorgan failure was an independent predictor of survival. In the index study, we did not find a correlation between the clinical causes of acute decompensation with short-term mortality. These results contrast with previously published data, and possible reasons include a small sample size.¹⁹ We also observed a close overlap of histologic features of acute hepatic insult

both in ACLF and in compensated CLD patients. Hepatic necrosis, the severity of lobular inflammation, and the absence of advanced hepatic fibrosis (Ishak stages 5–6) were histologic indicators of 28-day mortality in ACLF patients. Of these, dense lobular necroinflammatory activity was found to be a clinically independent histologic predictor of short-term mortality in ACLF patients on multivariate analysis. In other Indian studies, it was found that alcohol-associated ACLF and cryptogenic ACLF are phenotypically more severe than the ACLF related to other etiologies.²⁰

Published studies describing histologic features in liver biopsies from patients with ACLF are sparse, and the inclusion criteria vary among the available studies. Kato-nizadeh et al²¹ described histologic features of ACLF patients having chronic alcoholic cirrhosis, and ductular cholestasis and MDBs were reported as predictors of in-hospital mortality. Nacif et al⁹ in their study found that histologic evidence of cholestasis, along with Model for End-Stage Liver Disease score and total bilirubin, were higher in their ACLF patients than in other CLD patients. Rastogi et al⁸ described hepatic fibrosis stage ≥ 3 , ballooning, ductular proliferation, apoptosis, and amount of residual hepatic parenchyma as the indicators of poor outcome in a cohort of ACLF patients having mixed etiologies like our patients. They also classified the histologic findings as pattern I, having a poor prognosis, and pattern II, comprising hepatocyte ballooning, rosette formation, cholestasis, moderate to severe interface activity, and variable fibrosis, with a better outcome.⁸ In another

Table 3. Histopathologic Predictors of 28-Day Short-Term Mortality in Patients With Acute-on-Chronic Liver Failure (ACLF)

Histologic Parameters	Findings	Median (IQR) Survival, d	Hazard Ratio (95% CI)	P Value ^a
Diffuse hepatic necrosis	No	8 (6–12)	1	.01
	Yes	5 (3–8)	1.64 (1.11–2.41)	
Frequent apoptotic bodies	No	6 (4 to >28)	1	.56
	Yes	7 (6–10)	1.23 (0.62–2.42)	
Diffuse macrovesicular steatosis	No	9 (6–14)	1	.25
	Yes	6 (5–8)	1.25 (0.85–1.82)	
Diffuse microvesicular steatosis	No	7 (6–11)	1	.37
	Yes	7 (4–9)	1.23 (0.78–1.91)	
Severe cholestasis	No	5 (1 to >28)	1	.71
	Yes	7 (6–10)	1.25 (0.39–3.93)	
Cholestasis (ductular)	No	6 (5–9)	1	.21
	Yes	8 (6–13)	0.78 (0.54–1.14)	
Severe confluent necrosis	No	7 (6–10)	1	.12
	Yes	7 (5–11)	1.33 (0.92–1.93)	
Dense lobular inflammation (with prominent ceroid-laden macrophages)	No	8 (6–11)	1	.03 (multivariate analysis, .04)
	Yes	4 (2–5)	1.73 (1.04–2.87)	
Dense portal inflammation	No	8 (6–10)	1	.95
	Yes	6 (4–18)	1.01 (0.63–1.61)	
Dense interface hepatitis	No	7 (5–10)	1	.10
	Yes	7 (6–12)	0.71 (0.47–1.07)	
Prominent multinucleation	No	8 (1 to >28)	1	.46
	Yes	7 (6–10)	1.55 (0.49–4.86)	
Diffuse ballooned hepatocytes	No	2 (1 to >28)	1	.63
	Yes	7 (6–10)	0.76 (0.24–2.38)	
Prominent feathery degeneration	No	6 (5–8)	1	.08
	Yes	10 (7–13)	0.72 (0.50–1.03)	
Ishak HAI stages 5–6 (advanced)	No	4 (3–6)	1	.008 (multivariate analysis, .02)
	Yes	8 (7–11)	0.52 (0.32–0.84)	
METAVIR stages 3–4 (advanced)	No	4 (3–12)	1	.11
	Yes	8 (6–10)	0.64 (0.36–1.09)	

Abbreviations: HAI, histologic activity index; IQR, interquartile range.

^a Bold entries refer to statistically significant *P* values; bold cells indicate statistically significant parameters identified from multivariate analysis.

study by Jain et al,⁷ the authors showed pattern I histology was present in 76.9% of nonsurvivors, whereas pattern II histology was found in 94.3% of patients with a favorable prognosis, with little overlap. Li et al²² described a higher prevalence of multiple-organ failure and shorter patient survival in patients with hepatic necrosis. Our study did not find a clear statistically significant correlation between patients' survival and the histologic pattern I and pattern II class types. Also, contrary to the index study, Rastogi et al⁸ observed poor survival in patients with higher fibrosis stages, indicating less functional hepatocyte reserve. In our study, patients with biopsies having advanced hepatocyte fibrosis were found to have relatively longer survival than those without advanced documented hepatic fibrosis (Table 3). A similar trend was also noted with higher METAVIR fibrosis stages, but the difference was not statistically significant (*P* = .11). These contrasting features may indicate that the available viable pool of functional hepatocytes is more important than the amount of fibrosis in liver cores; however, these are speculative hypotheses, and a definite answer could have been provided on liver resections—also, however, the same is not indicated in these patients. Overall, looking at the overlapping histologic findings in

up to 28-day ACLF survivors and nonsurvivors, it seems inappropriate to classify the histologic features into 2 simplified patterns because the disease pathogenesis of ACLF and etiologies of acute hepatic decompensation vary and the exact mechanism is not fully understood. Some of these features were also identified in our compensated CLD patients without any feature of clinical decompensation, except hepatic necrosis. Moreover, the histologic features included in patterns I and II are based on qualitative grades and stages, hence making it subjective to imply in clinical reporting.

Although apart from establishing the histologic changes in patients with ACLF and bringing out a clinically independent histologic predictor of 28-day short-term mortality in patients with ACLF, the present study is limited by the nonuniformity of the number of liver biopsies included in different study groups, which was beyond our control. The number of 28-day ACLF survivors in this study was small (*n* = 25) in comparison with the ACLF nonsurvivors (*n* = 127). The liver biopsies of patients with ACLF and controls with disease were randomly selected based on clinical data availability and were not consecutive. Hence, the results can be affected by selection bias. Also, the cases

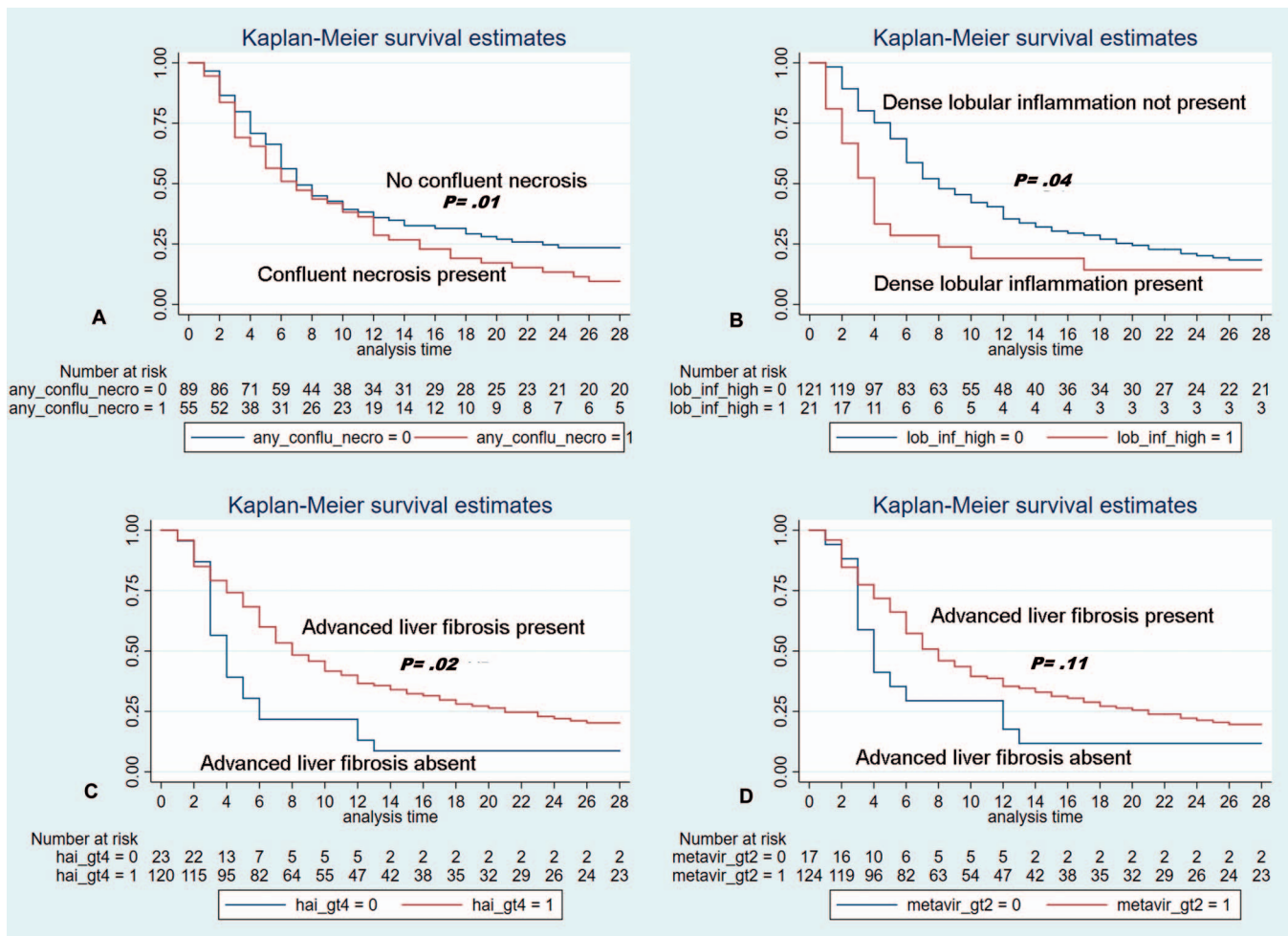


Figure 3. Kaplan-Meier curves showing differences of 28-day survival in patients with acute-on-chronic liver failure having confluent hepatic necrosis versus those who do not (A), having confluent dense lobular inflammation versus those who do not (B), having advanced Ishak fibrosis stages (stages 5 and 6) versus those who do not (C), and having advanced METAVIR fibrosis stages (stages 3 and 4) versus those who do not (D).

of compensated CLD included were not etiology- or stage-matched with ACLF patients. We did not have chronic alcoholic cirrhosis in the disease control group, whereas alcoholism was the most common cause of acute decompensation in our cohort of ACLF patients. The histologic features identified in core-needle biopsies may not be truly representative of the whole liver, and it is not the best modality to assess the volume of the residual functional liver; this study yet holds importance, keeping in mind the possible availability of prospective liver core biopsies from patients with ACLF in routine clinical practice. A transjugular liver biopsy or minilaparoscopic liver biopsy in patients with an inaccessible transjugular route is in the cards because of their relatively better safety features.² Liver biopsy in patients with ACLF hence helps to prognosticate patients objectively and stage the extent of liver fibrosis. It helps to differentiate between acute liver failure and ACLF in patients with previously undocumented CLD in a setting of clinical emergency.^{2,3} Also, in this study, liver biopsy helped to document the features of CLD in almost 35.8% of previously undocumented patients, and thus may be instrumental in differentiating acute liver failure from ACLF. Based on the existing knowledge and our findings, it

appears that pathologists must report liver biopsies in detail, mentioning the grades of lobular necroinflammatory activity and the extent of hepatocyte necrosis, and stage the hepatic fibrosis as discussed to help the clinicians predict the possible patients' outcome and organ failure.

To conclude, the clinical and histologic features in patients with ACLF in our cohort with divergent etiologies were distinctly more severe in ACLF than in patients with compensated CLD. Liver core biopsies in ACLF patients helped to establish the dense lobular necroinflammatory activity as a clinically independent histologic predictor of short-term mortality. A larger multi-institution-based evaluation of prognostic histologic features in ACLF patients may significantly help in clinical risk assessment.

References

- Jalan R, Yurdaydin C, Bajaj JS, et al. Toward an improved definition of acute-on-chronic liver failure. *Gastroenterology*. 2014;147(1):4–10.
- Arroyo V, Moreau R, Jalan R, et al. Acute-on-chronic liver failure: a new syndrome that will re-classify cirrhosis. *J Hepatol*. 2015;62(1):S131–S143.
- Sarin SK, Choudhury A. Acute-on-chronic liver failure: terminology, mechanisms and management. *Nat Rev Gastroenterol Hepatol*. 2016;13:131–149.
- Moreau R, Jalan R, Gines P, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology*. 2013;144(7):1426–1437.

5. Bajaj JS, O'Leary JG, Reddy KR, et al. Second infections independently increase mortality in hospitalized cirrhotic patients: the North American consortium for the study of end-stage liver disease (NACSELD) experience. *Hepatology*. 2012;56(6):2328–2335.
6. van Leeuwen DJ, Alves V, Balabaud C, et al. Acute-on-chronic liver failure 2018: a need for (urgent) liver biopsy? *Expert Rev Gastroenterol Hepatol*. 2018; 12(6):565–573.
7. Jain R, Jha A, Jain K, et al. Clinical and histologic markers of poor prognosis in acute on chronic liver failure (ACLF)—an Indian perspective. *Int J Sci Res*. 2018; 7(2).
8. Rastogi A, Kumar A, Sakhuja P, et al. Liver histology as predictor of outcome in patients with acute-on-chronic liver failure (ACLF). *Virchows Arch*. 2011; 459(2):121.
9. Nacif LS, Aquino F, Tanigawa RY, et al. Histopathologic evaluation of acute on chronic liver failure. *Transplant Proc*. 2020;52(5):1325–1328.
10. Meersseman P, Wilmer A. Evolving concepts in acute-on-chronic liver failure. *ICU Manage Pract*. 2016;16(3):144–147.
11. Pugh R, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg*. 1973;60(8):646–649.
12. Kamath PS, Wiesner RH, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology*. 2001;33(2):464–470.
13. Knaus WA, Draper EA, Wagner DP, et al. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13(10):818–829.
14. Ishak K, Baptista A, Bianchi L, et al. Histologic grading and staging of chronic hepatitis. *J Hepatol*. 1995;22:696–699.
15. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C: the METAVIR Cooperative Study Group. *Hepatology*. 1996;24:289–293.
16. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histologic scoring system for nonalcoholic fatty liver disease. *Hepatology*. 2005; 41(6):1313–1321.
17. Crawford JM, Burt AD. Anatomy, pathophysiology, and basic mechanisms of disease. In: Burt AD, Ferrell LD, Portmann BC, eds. *MacSwen's Pathology of the Liver*. 6th ed. London, UK: Churchill Livingstone; 2011:1–61.
18. Shalimar, Saraswat V, Singh SP, et al. Acute-on-chronic liver failure in India: the Indian National Association for Study of the Liver consortium experience. *J Gastroenterol Hepatol*. 2016;31(10):1742–1749.
19. Shalimar, Kedia S, Mahapatra SJ, et al. Severity and outcome of acute-on-chronic liver failure are dependent on the etiology of acute hepatic insults. *J Clin Gastroenterol*. 2017;51(8):734–741.
20. Ray G, Manjubhargav P. Clinical presentation and mortality determinants of alcohol-related liver disease: a single-center experience of the rising menace from eastern India. *Inflamm Intest Dis*. 2019;4(3):104–114.
21. Katoonizadeh A, Laleman W, Verslype C, et al. Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. *Gut*. 2010;59(11): 1561–1569.
22. Li H, Xia Q, Zeng B, et al. Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation. *J Hepatol*. 2015;63(1):50–59.