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

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- **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 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.

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A cute-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.6 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.9–12 In a study based on prospectively maintained ACLF cases 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.

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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. 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. The clinical details, including blood investigations, etiologic workup, and mortality data within 28 days of hospitalization, were obtained from a prospectively maintained database. 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

**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 comprised 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 Ishak histologic activity index (HAI) grading system. 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 co-operative study group staging system of liver fibrosis for hepatic fibrosis assessment. 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 the sepsis-related changes. Feathery degeneration was defined as swelling of hepatocytes with cytoplasmic rarefaction and the presence of cytoplasmic bile plug and neutrophils. The presence of large punched-out acidophil bodies, their 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. 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. Patterns of fibrosis, in the form of the portal, periportal, bridging, or pericellular fibrosis, were analyzed. Periportal fibrosis was defined by the presence of coarse parallel reticulin fibers in the periporal 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, and correlated with patient survival data.

**Statistical Analysis**
Data were presented as mean (±SD), median (interquartile range [IQR]), or frequency (percentage). For comparison of categoric variables, we applied the χ² test or Fisher exact test as appropriate. For continuous variables following the 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 which came 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

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

| Variable | 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 mm³ | 12 900 (8950–20 150) | 7300 (4745–8900) | <.001 |
| Platelets, ×10³/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 parenthesis 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%), 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). Macronodular 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 B virus infection, acute hepatitis A virus infection, viral reactivation, acute autoimmune event, and variceal bleed in 1 of 30 cases (3.33%) each. Most common chronic etiologies associated with microvesicular steatosis were alcohol (12 of 30; 40%), chronic viral hepatitis (7 of 30; 23.3%), 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. Mallory Denk bodies were identified in 69 of 148 liver biopsies (50.67%) from ACLF (Figure 2, C and D). Dense portal tract inflammation involving most of the portal tracts was noted in 34 of 28 biopsies (18.5%), predominantly comprising lymphocytes (Figures 1, A and B, and 2, E and F). Diffuse interface hepatitis involving all portal tracts was noted in 110 of 149 (59.7%; Figure 2, A, and 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 B virus infection, acute hepatitis A virus infection, viral reactivation, acute autoimmune event, and variceal bleed in 1 of 30 cases (3.33%) each. Most common chronic etiologies associated with microvesicular steatosis were alcohol (12 of 30; 40%), chronic viral hepatitis (7 of 30; 23.3%), 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. Mallory Denk bodies 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). 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). 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).

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* |
|-----------------------|--------------------------|-----------------------------------|----------|
| 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| 72/148 (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 (66.61)         | 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 (60.0)    | 23/98 (23.47)                    | <.001    |
| Multinucleation       | 144/148 (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 (40.67)           | 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.

* Bold entries refer to statistically significant P values.

Comparison of Histologic Features Between ACLF and Compensated CLD

Liver biopsies from patients with ACLF in comparison with the compensated CLD 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 was found as an independent predictor of short-term mortality.
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 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, microvascular 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 histological 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). Especially, the severity of lobular inflammation and hepa-
tocyte 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. 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. 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 the alcohol-associated ACLF and cryptogenic ACLF are phenotypically more severe than the ACLF related to other etiologies.

Published studies describing histologic features in liver biopsies from patients with ACLF are sparse, and the inclusion criteria vary among the available studies. Katonizadeh et al 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 al 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 al 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. In another study by Jain et al, 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 al\(^{22}\) 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 al\(^{8}\) 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 varies 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 the 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 of compensated CLD included were not etiology- or stage-matched with ACLF patients. We did not have chronic

| Histologic Parameters                              | Findings | Median Survival, d (IQR) | Hazard Ratio (95% CI) | \(P\) Value* |
|---------------------------------------------------|----------|--------------------------|-----------------------|--------------|
| Diffuse hepatic necrosis                          | No       | 8 (6–12)                 | 1                     | .01          |
|                                                   | Yes      | 5 (3–8)                  | 1.64 (1.11–2.41)      | .56          |
| Frequent apoptotic bodies                         | No       | 6 (4 to >28)             | 1                     | .25          |
|                                                   | Yes      | 7 (6–10)                 | 1.23 (0.62–2.42)      | .37          |
| Diffuse macrovesicular steatosis                  | No       | 9 (6–14)                 | 1                     | .71          |
|                                                   | Yes      | 6 (5–8)                  | 1.25 (0.85–1.82)      | .21          |
| Diffuse microvesicular steatosis                  | No       | 7 (6–11)                 | 1                     | .12          |
|                                                   | Yes      | 7 (4–9)                  | 1.23 (0.78–1.91)      | .03 (multivariate analysis, .04) |
| Severe cholestasis                                | No       | 5 (1 to >28)             | 1                     | .11          |
|                                                   | Yes      | 7 (6–10)                 | 1.25 (0.39–3.93)      | .11          |
| Cholestasis (ductular)                            | No       | 6 (5–9)                  | 1                     | .95          |
|                                                   | Yes      | 8 (6–13)                 | 0.78 (0.54–1.14)      | .10          |
| Severe confluent necrosis                         | No       | 7 (6–10)                 | 1                     | .46          |
|                                                   | Yes      | 7 (5–11)                 | 1.33 (0.92–1.93)      | .08          |
| Dense lobular inflammation (with prominent ceroid-laden macrophages) | No | 8 (6–11) | 1 | .008 (multivariate analysis, 0.02) |
|                                                   | Yes      | 4 (2–5)                  | 1.73 (1.04–2.87)      | .008         |
| Dense portal inflammation                        | No       | 8 (6–10)                 | 1                     | .63          |
|                                                   | Yes      | 6 (4–18)                 | 1.01 (0.63–1.61)      | .10          |
| Dense interface hepatitis                         | No       | 7 (5–10)                 | 1                     | .46          |
|                                                   | Yes      | 7 (6–12)                 | 0.71 (0.47–1.07)      | .10          |
| Prominent multinucleation                         | No       | 8 (1 to >28)             | 1                     | .03 (multivariate analysis, .04) |
|                                                   | Yes      | 7 (6–10)                 | 1.55 (0.49–4.86)      | .11          |
| Diffuse ballooned hepatocytes                     | No       | 2 (1 to >28)             | 1                     | .08          |
|                                                   | Yes      | 7 (6–10)                 | 0.76 (0.24–2.38)      | .11          |
| Prominent feathery degeneration                   | No       | 6 (5–8)                  | 1                     | .008         |
|                                                   | Yes      | 10 (7–13)                | 0.72 (0.50–1.03)      | .08          |
| Ishak HAI stages 5–6 (advanced)                   | No       | 4 (3–6)                  | 1                     | .11          |
|                                                   | Yes      | 8 (7–11)                 | 0.52 (0.32–0.84)      | .11          |
| Metavir stages 3–4 (advanced)                     | No       | 4 (3–12)                 | 1                     | .11          |
|                                                   | Yes      | 8 (6–10)                 | 0.64 (0.36–1.09)      | .11          |

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

* Bold entries refer to statistically significant \(P\) values; bold cells indicate statistically significant parameters identified from multivariate analysis.
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. 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. 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 histological features in patients with ACLF in our cohort with divergent etiologies were distinctly 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 histological predictor of short-term mortality. A larger multi-institution-based evaluation of prognostic histological features in ACLF patients may significantly help in clinical risk assessment.

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