# CLINICAL PARAMETERS ARE MORE PREDICTIVE OF MORTALITY IN ALCOHOLIC HEPATITIS THAN HISTOPATHOLOGIC SEVERITY

by

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DISSERTATION

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# ABSTRACT
# CLINICAL PARAMETERS ARE MORE PREDICTIVE OF MORTALITY IN ALCOHOLIC HEPATITIS THAN HISTOPATHOLOGIC SEVERITY

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**Background**: Alcoholic hepatitis (AH) is primarily diagnosed by clinical parameters, but is often misdiagnosed due to nonspecific symptoms, leading to high mortality rates. While histology aids definitive diagnosis, the role of the liver biopsy in its workup is still controversial. Currently, there is no widely accepted grading histologic grading system for AH. The relationship between biopsy findings and clinical course is also yet unknown. The Alcoholic Hepatitis Histologic Score (AHHS) was recently developed to define patient prognosis by histologic criteria.

**Objective**: The purpose of this study was to compare histologic severity defined by the AHHS with clinical severity of AH, as seen with symptoms, laboratory markers, and patient survival.

**Methods**: We conducted a retrospective case series of 56 patients with biopsy-proven AH from two hospitals in Dallas, TX, USA. Clinical and demographic data were collected from electronic medical records. Two trained pathologists blinded to patients' outcomes graded liver biopsies using the AHHS criteria. Relationships between clinical symptoms and complications, laboratory investigations, patient outcomes, individual histologic

features, and the AHHS were analyzed.

**Results**: No hematologic or biochemical laboratory markers significantly correlated with the AHHS. Higher AST correlated with a greater degree of steatosis on biopsy ($p<0.0019$). Severe neutrophil infiltration on biopsy correlated with higher serum bilirubin, INR, MELD, and DF ($p=0.034$). Survival analysis by Kaplan-Meier curves and log-rank tests showed no significant correlation between AHHS and 90-day survival ($p=0.09$), while multiple clinical scoring systems accurately stratified prognosis ($p<0.018$ for all). Severe neutrophil infiltration on biopsy also correlated significantly with death ($p=0.0001$).

**Conclusion**: Retrospective analysis in a diverse U.S. urban cohort did not confirm the validity of AHHS to predict survival in AH. In contrast, clinical parameters were better predictors of survival. Our results suggest that clinical deterioration, rather than histopathologic severity, is more informative in determining prognosis in AH. The relationship between neutrophil infiltration and mortality deserves further study.

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# PRIOR PUBLICATIONS & PRESENTATIONS

PUBLICATIONS:

Yeluru, A., et al. (2016). "Alcoholic Hepatitis: Risk Factors, Pathogenesis, and Approach to Treatment." Alcohol Clin Exp Res 40(2): 246-255.

PRESENTATIONS AND POSTERS:

Dean's Research Scholars final presentation. "Correlation of Biomarkers with Histopathologic Severity of Alcoholic Hepatitis". March 11, 2015. UT Southwestern Medical Center.

MD Distinction in Research final presentation. "Mortality in Alcoholic Hepatitis: Clinical vs. Histologic Severity". February 16, 2016. UT Southwestern Medical Center.

# CHAPTER 1
# INTRODUCTION

Alcoholic hepatitis (AH) is a severe systemic illness resulting from alcohol injury to the liver and is the most severe form of decompensated alcoholic liver disease (ALD). Presentation can be non-specific, and patients often have complications such as variceal bleeding and infections, further decreasing the diagnostic accuracy. Alcoholic hepatitis was misdiagnosed in 10-50% of patients across several studies when relying solely on clinical parameters such as presence of jaundice and elements of the systemic inflammatory response syndrome (SIRS) (Mookerjee 2011, Elphick 2007, Forrest 2012). Liver biopsy might lead to a more certain diagnosis, but a timely biopsy and pathology consultation is not uniformly accessible. A transjugular biopsy is the preferred method in these patients, because severe coagulopathy and ascites are often present. Liver biopsy typically shows the acute features of steatosis, neutrophilic inflammation, hepatocyte ballooning degeneration, and Mallory bodies, along with more chronic features of fibrosis and cholestasis of varying degrees (Elphick 2007, Altamirano 2014). For unclear reasons, liver biopsies are more commonly performed in Europe in patients with suspected AH; in the United States, clinical diagnosis is generally accepted.

There are limited studies examining a correlation between histologic severity and clinical presentation and outcomes (Mookerjee 2011, Altamirano 2014). One such study from Europe was published by Altamirano et al in 2014, in which they developed a prognostic model based on histologic features of AH, called the Alcoholic Hepatitis

Histologic Score (AHHS). The model was developed in a cohort of 217 patients and validated in a separate cohort of 109 patients. Of all the histologic features studied, four were significantly associated with 90-day mortality in univariate analysis: stage of fibrosis, bilirubinostasis, neutrophil infiltration, and megamitochondria. These four features were combined into a scoring model, which predicted 90-day mortality with an area under the receiver operating characteristic value of 0.77 (95% CI, 0.71-0.83).

The pathogenesis of alcoholic hepatitis is not completely defined. Hepatic inflammation triggered by an increase in gut permeability is considered to be a hallmark of the disease (Yeluru 2016). Consequently, treatment for AH relies upon systemic anti-inflammatory therapy in addition to supportive measures. Recent studies have demonstrated that patients respond differently to anti-inflammatory therapy (Louvet 2007). If there are histologic variants in alcoholic hepatitis with implications for therapy or outcome, then a liver biopsy may become standard of care for suspected AH. To date, sub-types of AH have not been defined. Thus, the aim of our study was to find correlations between clinical severity in patients with AH and histological severity as defined by the AHHS and to predict survival in an ethnically diverse population.

# CHAPTER 2
# PATIENTS AND METHODS

*Study Cohort (see figure 1)*

Using an Institutional Review Board-approved protocol, Parkland Hospital and Health System (PHHS) and University Hospital St. Paul (UHSP) electronic medical record databases were queried for patients matching the following inclusion criteria: (1) diagnosis of alcohol-related liver diseases as coded by ICD-9, and (2) liver biopsy. A total of 286 patients had a liver biopsy and a concurrent ICD-9 diagnosis code of alcoholic liver disease between January 2005 and January 2015. After detailed medical record review, 104 were excluded for concomitant viral hepatitis, 47 for presence of malignancy, and 21 for other liver diseases or confounding clinical variables, such as HIV infection, heterozygous alpha-1 anti-trypsin deficiency, possible drug-induced liver injury, probable non-alcoholic fatty liver disease, or having aspartate aminotransferase (AST) levels lower than alanine aminotransferase (ALT) on admission. AST greater than ALT is a hallmark sign of alcohol-related liver disease (Nalpas 1984).

Of those remaining with isolated alcoholic liver disease (n = 114), 37 with alternate pathologic diagnoses, 19 with their last drink more than 2 months prior to biopsy, and 2 with AST >500 U/L were also excluded from further study. Alternate histology included nonspecific inflammation without steatosis or ballooning, necrosis with parenchymal collapse, ductular proliferation, interface hepatitis, congestive hepatopathy, and bland cirrhosis. The remaining 56 patients met the inclusion criteria:

documented alcohol use within 2 months of liver biopsy and a histologic diagnosis of alcoholic steatohepatitis.

*Data collection*

Clinical data collected included: age at diagnosis, sex, race, duration and amount of alcohol intake, presenting symptoms, presence of complications at admission (encephalopathy, variceal bleeding, ascites, or infection), reason for biopsy, length of AH episode, survival at 28 days, 90 days, and 180 days, discharge disposition, imaging, treatment, vital signs on admission, and laboratory investigations. Data were censored at last follow-up for those patients who were lost to follow-up before 6 months. Modified Maddrey discriminant function (DF), Model of End-stage Liver Disease (MELD), and ABIC (age bilirubin INR creatinine), and Systemic Inflammatory Response Syndrome (SIRS) scores were calculated (Maddrey 1978, Bone 1992, Kamath 2001, Dominguez 2008). Liver biopsy specimens stained in hematoxylin and eosin (H&E) were graded by two gastrointestinal pathologists (L.P., P.G.). The pathologists trained using the AHHS virtual training slides provided by Dr. John Woosley, AHHS lead pathologist. Slides were graded separately, and subsequently consensus was reached on specimens with initial scoring disagreement. Histologic features analyzed include: level of steatosis, ballooning, Mallory bodies, neutrophil inflammation, bilirubinostasis, stage of fibrosis, and presence of megamitochondria. Both pathologists were blinded to the patients' clinical course.

*Statistical Analysis*

Continuous variables are described as means ± standard deviations. Categorical variables are described as percentages. Clinical and histologic data from the local cohort are compared with that from the AHHS training cohort. The AHHS paper reported its data as medians and interquartile ranges (Altamirano 2014). However, since there is no established method for comparing medians and interquartile ranges of two populations, Student's t-test was performed using local mean ± standard deviation and the AHHS median as "population mean". Continuous variables were compared with unpaired t-tests; logarithmic transformation was used for non-normal distributions. For categorical variables, the chi-squared test was used. To find correlations between AST and biopsy findings, AST was dichotomized into two categories: AST<80 and AST>80. AST of 80 was chosen as the dichotomization point because AST<80 was an exclusion criteria in a current U01 clinical trial for the treatment of alcoholic hepatitis (clinicaltrials.gov). Associations between two continuous variables were analyzed by the Spearman correlation coefficient. All p-values are two-tailed. Survival up to 180 days was analyzed with Kaplan-Meier survival curves, and significance was calculated using the log-rank test. Two patients who received liver transplant as part of their treatment of AH were censored from survival analysis at the time of transplantation. All calculations were performed in STATA/SE 13.0.

# CHAPTER 3
# RESULTS

## *Patient Demographics*

Demographics and clinical information on the 56 patients in the cohort are summarized in Table 1, with data from this set being compared to the training cohort from Altamirano et al (Altamirano 2014). Overall, there were no significant differences in the demographics between the two cohorts. The local cohort was ethnically diverse, with 52% non-Hispanic white, 36% Hispanic, 7% African American and 5% Asian/Pacific Islander. Laboratory results are summarized in Table 2. Laboratory values that were significantly different between the two cohorts included platelet count, ALT, sodium, creatinine, and MELD (all $p \leq 0.03$). The higher MELD score ($p=0.027$) in the local cohort may suggest that on average, local patients have more clinically severe AH than those from the AHHS cohort. For treatment, 35 patients (63%) received only supportive care, 9 patients (16%) received some corticosteroids, 6 patients (11%) received pentoxifylline, and the remaining 11% received liver transplant or other treatments. Of note, no patient in this cohort finished the prescribed course of steroids (28 days). Some received a 5-day course while steroids were discontinued in others due to suspected infection.

## *Biopsy findings*

Histologic features on biopsy are summarized in Table 3. Liver biopsy was pursued for diagnosis in 85% of patients, and for prognosis in 15% of patients. Of the seven biopsy features studied, four constitute the AHHS: stage of fibrosis,

bilirubinostasis, neutrophil inflammation, and megamitochondria (Altamirano 2014). The local cohort had more patients with lower levels of steatosis (p=0.007), ballooning (p=0.001), Mallory bodies (p=0.019) and megamitochondria (p=0.009) than the AHHS patients. Of these four histologic features, the presence of megamitochondria is the only one that is part of the AHHS. Overall, 54% of the cohort were in the severe category (n = 30, AHHS 6-9), 38% moderate (n = 21, AHHS 4-5), and 9% mild (n = 5, AHHS 0-3). The AHHS category proportions were not statistically different between the local cohort and the AHHS initial cohort (p=0.61) nor with the AHHS validation cohort (p=0.083).

*Clinical and histological correlations*

There were no significant correlations between any clinical symptom present on admission (ascites, encephalopathy, varices, infection) and the AHHS (p>0.1). In contrast, both DF and MELD correlated with ascites and encephalopathy, but not varices or infection, also supporting a more advanced stage of liver disease. There were no significant correlations between any laboratory marker (e.g. AST) and the AHHS (p>0.1). Higher AST correlated with higher levels of steatosis (p=0.0011). There was a trend towards correlation of higher AST with ballooning (p=0.062) on biopsy. Higher degree of neutrophil infiltration correlated with higher bilirubin (p=0.034) and higher MELD (p=0.025). No other significant correlations were found between any other clinical symptom, laboratory marker, or choice of treatment and individual histologic features.

*Survival analysis*

In the cohort of 56 patients, there were 7 deaths within 90 days (13% mortality), 8 lost to follow-up, and 2 patients who underwent liver transplant. There were no deaths between 90 and 180 days. Figure 2 shows the Kaplan-Meier curves categorized by AHHS. There were more deaths in the AHHS moderate category (n=5) than in the severe category (n=2). There was no significant difference in mortality rates among the three AHHS categories (p=0.092, log-rank test). In contrast, MELD (<21 vs. ≥21), DF (<32 vs. ≥32), and ABIC classifications all demonstrated significant differences in mortality (all p<0.02), as shown in Table 4. Finally, survival by severity of individual histologic features was also analyzed. Of the seven histologic features that the pathologists graded, the severity of neutrophil infiltration was significantly associated with mortality; higher neutrophil infiltration predicted higher mortality rates (p=0.0001, log-rank test). Six of the seven patients who died had severe neutrophil infiltration on biopsy. Less steatosis on biopsy also correlated with mortality (7/7 deaths, p=0.049). None of the other histologic features had significant association with mortality by log-rank test (Table 5). As indicated, the two patients who had liver transplants were censored from survival analysis at the time of transplantation.

*Systemic Inflammatory Response Syndrome (SIRS)*

45% of the cohort met the definition for SIRS on admission, fulfilling at least two SIRS criteria. Six of the seven patients who died in this cohort had SIRS on admission. Survival analysis for presence and absence of SIRS was statistically significant by log-

rank test ($p=0.018$). An abnormal temperature using SIRS criteria, present in 6 out of the 7 deaths, was associated with mortality ($p<0.0001$) as was the number of SIRS criteria met ($p=0.0008$). No statistically significantly relationship was found between meeting SIRS criteria and the prognostic markers such as MELD, DF, ABIC and AHHS, nor between meeting SIRS criteria and biopsy features. In addition, there was no statistically significant relationship between patients with positive SIRS and presence of infection ($p=0.095$).

## CHAPTER 4
## DISCUSSION AND CONCLUSIONS

The purpose of this study was to explore relationships between markers of clinical severity and histopathological severity in patients with biopsy-proven alcoholic hepatitis, and relate them to patient prognosis. The AHHS was the second study of its kind to prognosticate AH using histologic features (Altamirano 2014); Mookerjee et al in their 2011 study used different criteria. The studies differ considerably from each other and neither study has been validated to date. Currently, severity of AH is primarily defined by clinical prognostic scores, such as DF, MELD, and ABIC (Forrest 2005, Srikureja 2005, Dominguez 2008). Recent studies have also shown the striking role of SIRS: the presence of SIRS correlates with a much higher mortality rate in patients with AH (Mookerjee 2011, Michelena 2015).

The results of this study show that clinical prognostic calculators including DF, MELD and ABIC reliably predicted 90-day mortality, whereas the AHHS did not. Based on these results, the severity of the clinical syndrome, as seen by rising bilirubin, acute kidney injury, and coagulation abnormalities, is more directly applicable to patient prognosis than the histopathologic severity of alcoholic steatohepatitis. Definitive histologic grading may be of use in research, as active investigation into the pathophysiology of AH and translational trials seek targeted therapies. However, as there is no consistent relationship between histologic alcoholic steatohepatitis and clinical AH, it is yet unclear whether the cost and risk of complications of a liver biopsy are justified

in the clinical setting. This issue was also explored by the Steroids or Pentoxifylline for Alcoholic Hepatitis (STOPAH) trial, a recent large multi-center randomized control trial evaluating prednisolone and pentoxifylline for AH treatment. The investigators chose to not require biopsy-proven AH as an inclusion criterion in an effort to best simulate clinical practice, even though this reduced the homogeneity of the patient population.

The distinction between acute and chronic markers of alcoholic liver injury is necessary. Steatosis, ballooning, neutrophil inflammation, and megamitochondria are seen in the acute phase of decompensated ALD (Uchida 1984, Elphick 2007), while fibrosis, cholestasis, and lack of neutrophils are more longitudinal features. There is mixed evidence on whether acute features on histology are more predictive of survival than chronic features (Mookerjee 2011, Altamirano 2014, Elphick 2007). Mookerjee et al reported that the acute features are more predictive of prognosis, whereas Altamirano et al found the absence of acute features and the presence of chronic features to be more predictive of prognosis. In the current study, the only histologic feature that correlated with survival was the severity of neutrophil infiltration: six out of seven patients who died had severe neutrophil infiltration, and this finding was significant by log-rank test ($p=0.0002$). Similar findings were reported in prior studies (Marra and Tacke 2014). More studies are necessary to determine whether baseline hepatic function drives mortality rather than the severity of alcoholic injury in patients with alcoholic hepatitis.

AST has long been central for diagnosis for AH, but its role in prognosis is inconclusive. AST is used in the decision as to whether a patient's liver disease is alcoholic vs. non-alcoholic: an AST/ALT ratio of greater than 2 is suggestive of ALD (Cohen and Kaplan 1979). AST is released by damaged hepatocytes, and specifically in ALD, it is postulated that AST is released from mitochondria in response to direct alcohol-induced toxicity (Nalpas 1984). In our study, AST correlated positively with levels of steatosis and ballooning on histology, both features of acute alcoholic injury to hepatocytes (Elphick 2007). To our knowledge, this is the first study to report a direct correlation between AST, a clinical marker of alcoholic hepatocyte injury, with steatosis and ballooning, histologic markers of alcoholic hepatocyte injury. Overall, however, AST did not significantly correlate with histologic severity calculated by AHHS, clinical prognostic scores, or 90-day survival. Thus, AST may be a marker for acute alcoholic injury to the liver, but this relationship must be further studied prior to using it as a reliable inclusion or exclusion criterion for clinical trials.

Of note, the two pathologists had high initial concordance rates on all biopsy features except for megamitochondria, which has significant weight in the AHHS. Megamitochondria develop in response to ethanol-induced free radical formation (Uchida 1984). One study reported a higher prevalence of megamitochondria in livers with ALD (27.8%) vs. non-alcoholic liver disease (0.7%) (Uchida 1984). Uchida et al also reported higher frequency of megamitochondria in liver with more alcoholic foamy degeneration than other patterns of ALD. Regardless of its specificity for ALD, the presence of

megamitochondria is not routinely assessed by pathologists in diagnosing ALD or AH on liver biopsy. Thus, the lack of reliability of this criterion may be a limitation in calculating AHHS.

Our study was unable to validate the AHHS in a diverse American cohort. There are several factors that may limit the validity of our results. First, there is a potential selection bias in this study. In the AHHS study, liver biopsy was performed in all patients consecutively admitted for suspected alcoholic hepatitis, as is common in certain specialized hospitals in Europe. In contrast, in the United States, biopsy is not routinely performed in cases of suspected AH; biopsy is only pursued for patients whose diagnosis is uncertain or confusing. Thus, the patients selected in this study may not be representative of all patients presenting with alcoholic hepatitis. Second, the timing of the biopsy is not consistent across all patients. Some patients were biopsied in early admission prior to beginning disease-modifying treatment, whereas others underwent biopsy several weeks after. Treatment with corticosteroids may change the degree and nature of the inflammatory infiltration in the liver, although this was not found in the current study, and likely also has an effect on the hospital course of the patient.

In conclusion, our data suggest that clinical parameters, such as MELD score and SIRS criteria, fare better in assessing prognosis in alcoholic hepatitis, rather than histologic parameters. While liver biopsy may have a role in standardizing clinical trials and investigating histologic variants of AH with different treatment needs, routine liver

biopsy may not be necessary in treating majority of patients with AH. Future studies should focus on determining whether patient outcomes depend more on acute alcoholic injury or baseline hepatic function, as this has direct implications for treatment protocols.

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**Table 1.** Comparison of clinical demographics: No significant differences between the local cohort and the AHHS cohort.

| Parameter | Local cohort (n = 56) | Local cohort (n = 56) | AHHS cohort (n = 121) | p-value |
|---|---|---|---|---|
| Age (y) | 48 (40 – 57) | 48 ± 11 | 49 (41 – 54) | 0.49 |
| Male | 34 (61%) | | 78 (67%) | 0.37 |
| Alcohol intake† (g/day) | 84 (56 – 126) | 112 ± 87** | 100 (100 – 120) | 0.21 |
| Ethnicity | | | | |
| Non-Hispanic White | 54% | | | |
| Hispanic | 34% | | | |
| African American | 7% | | | |
| Asian/Pacific Islander | 5% | | | |
| Use of corticosteroids, n | 9 (17%) | | 54 (45%) | |
| Clinical decompensation at admission (n, %) | | | | |
| Ascites | 38 (68%) | | 82 (68%) | 1 |
| Variceal bleeding | 8 (14%) | | 26 (21%) | 0.27 |
| Encephalopathy | 5 (9%) | | 17 (14%) | 0.35 |
| Infection | 12 (21%) | | 18 (15%) | 0.26 |

Data displayed as median (interquartile range), mean ± standard deviation, or proportions (%).
** Non-normal data, compared after logarithmic transformation
† Local data available for n=45

**Table 2.** Comparison of laboratory values

| Parameter | Local cohort | Local cohort* | AHHS cohort n = 121 | p-value |
|---|---|---|---|---|
| Hb (g/dL)<br>Hct (calculated for AHHS), n = 56 | 33 (29 – 37) | 33 ± 6 | 11 (9 – 12)<br>33 (27 – 36) | 0.43 |
| Leukocyte count (x$10^9$/L), n = 56 | 9.3 (6.0 – 12.9) | 10.5 ± 6.8** | 8.9 (6.3 – 13.9) | 0.92 |
| Platelet count (x$10^9$/L), n = 55 | 150 (97 – 229) | 175 ± 109** | 121 (71 – 175) | 0.03 |
| AST (U/L), n = 55 | 113 (70 – 180) | 131 ± 77** | 125 (82 – 188) | 0.16 |
| ALT (U/L), n = 55 | 42 (26 – 56) | 50 ± 50** | 51 (35 – 71) | 0.0035 |
| Sodium† (mmol/L), n = 56 | 132 (129 – 135) | 131 ± 7** | 134 (130 – 137) | 0.0019 |
| Albumin† (g/dL), n = 55 | 2.8 (2.3 – 3.3) | 2.8 ± 0.7 | 2.7 (2.3 – 3.0) | 0.33 |
| Creatinine† (mg/dL), n = 56 | 0.84 (0.5 – 1.08) | 1.07 ± 1.04** | 0.8 (0.60 – 1.0) | 0.64 |
| Bilirubin† (mg/dL), n = 55 | 7.2 (2.4 – 22) | 11.6 ± 11.0** | 9.7 (4.3 – 17.7) | 0.20 |
| INR, n = 54 | 1.5 (1.3 – 2.0) | 1.7 ± 0.5 | 1.6 (1.4 – 1.9) | 0.92 |
| Maddrey modified DF, n = 54 | 36 (18 – 51) | 37 ± 27 | | |
| MELD, n = 54 | 21 (15 – 26) | 21 ± 8 | 18 (12 – 22) | 0.027 |
| Glasgow, n = 54 | 7 (6 – 8) | 7 ± 2 | | |
| ABIC score, n = 54 | 7.3 (6.0 – 8.4) | 7.4 ± 1.6 | 7.3 (6.6 – 8.4) | 0.51 |
| ABIC class, n = 54<br>A (< 6.71)<br>B (6.71 – 8.99)<br>C (≥ 9) | | <br>21 (39%)<br>24 (44%)<br>9 (17%) | <br>33 (27%)<br>68 (56%)<br>20 (17%) | 0.27 |

* Mean ± SD, same data as median and interquartile range
** Non-normal data, compared after logarithmic transformation
† Local cohort = plasma; AHHS = serum

**Table 3.** Histological findings

| **Parameter** | **AHHS points** | **Local cohort n = 56** | **AHHS cohort n = 121** | | **p-value** |
|---|---|---|---|---|---|
| Steatosis | | | | | |
| < 33% | | 32 (57%) | 39 (32%) | | 0.007 |
| 33% - 66% | | 11 (20%) | 35 (29%) | | |
| > 66% | | 13 (23%) | 47 (39%) | | |
| Ballooning | | | | | |
| Occasional | | 37 (66%) | 48 (40%) | | 0.001 |
| Marked | | 19 (34%) | 73 (60%) | | |
| Mallory bodies | | | | | |
| No or occasional | | 8 (14%) | 4 (3%) | | 0.019 |
| Marked | | 48 (86%) | 117 (97%) | | |
| Stage of fibrosis | | | | | |
| No fibrosis or portal fibrosis | 0 | 0 (0%) | 3 (2%) | | 0.31 |
| Expansive fibrosis | 0 | 6 (11%) | 19 (16%) | | |
| Bridging fibrosis or cirrhosis | +3 | 50 (89%) | 99 (82%) | | |
| Bilirubinostasis | | | | | |
| None | 0 | 19 (34%) | 37 (31%) | | 0.89 |
| Hepatocellular | 0 | 6 (11%) | 14 (12%) | | |
| Canalicular and/or ductular | +1 | 12 (21%) | 32 (26%) | | |
| Hepatocellular + canalicular and/or ductular | +2 | 19 (34%) | 38 (31%) | | |
| PMN infiltration | | | | | |
| No/Mild | +2 | 42 (75%) | 81 (67%) | | 0.31 |
| Severe | 0 | 14 (25%) | 40 (33%) | | |
| Megamitochondria | | | | | |
| No | +2 | 25 (45%) | 30 (25%) | | 0.009 |
| Yes | 0 | 31 (55%) | 91 (75%) | | |
| AHHS categories | | | *Initial* | *Validation** | 0.61 (Initial) 0.083 (Validation) |
| Mild (0 – 3) | | 5 (9%) | 30 (14%) | 17 (16%) | |
| Moderate (4 – 5) | | 21 (38%) | 80 (37%) | 24 (22%) | |
| Severe (6 – 9) | | 30 (54%) | 107 (49%) | 68 (62%) | |

* n = 109

**Table 4.** Log-rank survival analysis for prognostic scores associated with 90-day survival.

| Individual results | p value | Grouped | p-value |
|---|---|---|---|
| AHHS | 0.48 | AHHS mild, moderate, severe | 0.09 |
| MELD | < 0.0001 | MELD, <21, ≥ 21 | 0.0054 |
| DF | < 0.0001 | DF, <32, ≥ 32 | 0.017 |
| ABIC | < 0.0001 | ABIC < 6.71, 6.71 – 9, > 9 | 0.018 |
| SIRS criteria | 0.0008 | SIRS positive | 0.018 |

**Table 5.** Log-rank survival analysis for individual histologic features: Mortality was significantly higher in patients with severe neutrophil infiltration compared to those with none-mild infiltration. No other histologic feature was associated with mortality.

| Feature | log-rank p-value |
|---|---|
| Steatosis | 0.049 |
| Ballooning | 0.26 |
| Mallory bodies | 0.97 |
| PMN | 0.0001 |
| Megamitochondria | 0.83 |
| Fibrosis | 0.38 |
| Bilirubinostasis | 0.10 |

## LIST OF FIGURES

**Figure 1.** Patient selection

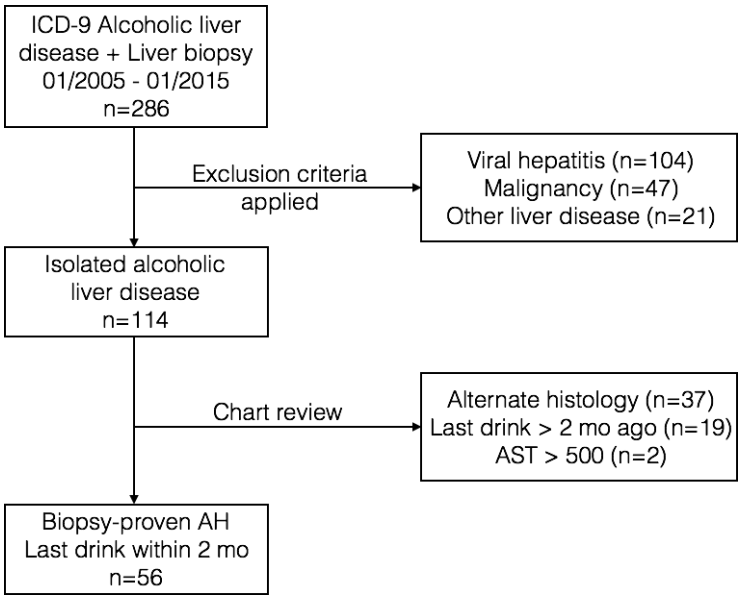
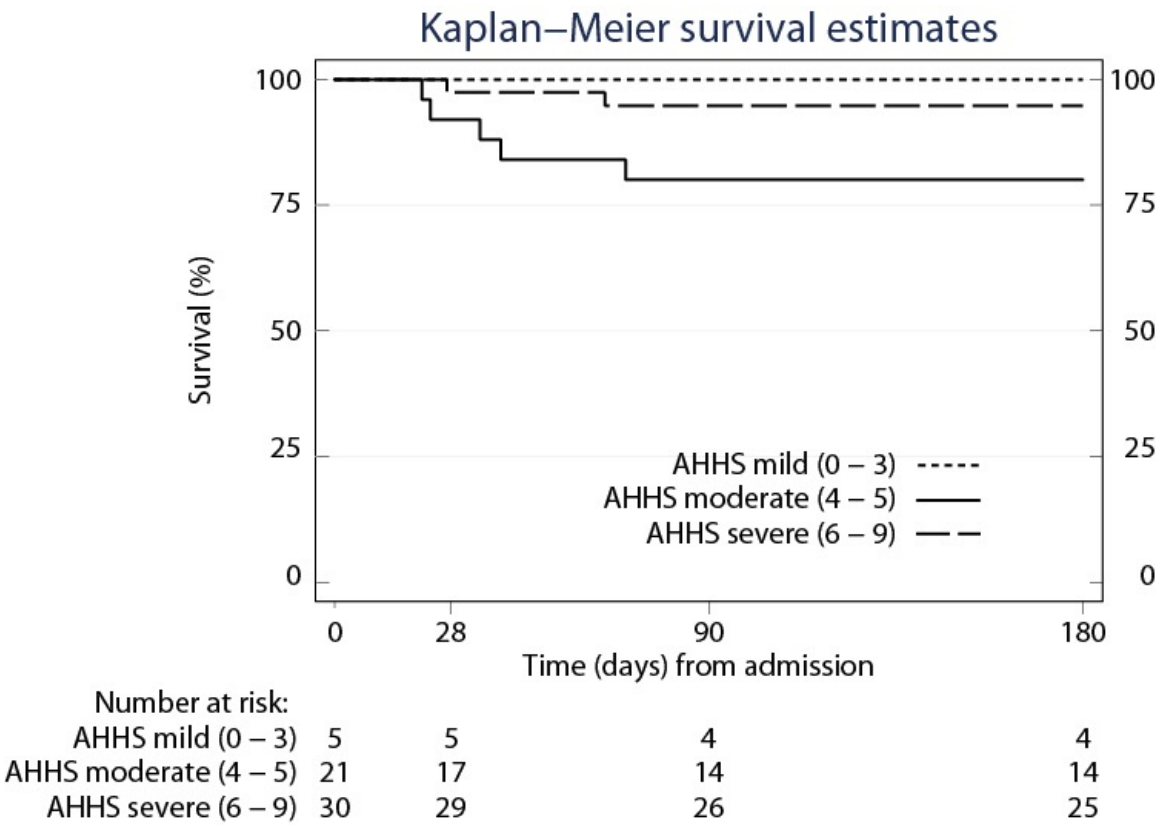


**Figure 2:** A Kaplan-Meier plot demonstrating the lack of association between AHHS prognostication and 180-day survival (log-rank: p=0.09)




| Number at risk: | | | | |
|---|---|---|---|---|
| AHHS mild (0 – 3) | 5 | 5 | 4 | 4 |
| AHHS moderate (4 – 5) | 21 | 17 | 14 | 14 |
| AHHS severe (6 – 9) | 30 | 29 | 26 | 25 |

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**REFERENCES**

Altamirano, J., et al. (2014). "A histologic scoring system for prognosis of patients with alcoholic hepatitis." Gastroenterology 146(5): 1231-1239 e1231-1236.

Ble, M., et al. (2014). "Transjugular liver biopsy." Clin Liver Dis 18(4): 767-778.

Bone, R. C., et al. (1992). "Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine." Chest 101(6): 1644-1655.

Cohen, J. A. and M. M. Kaplan (1979). "The SGOT/SGPT ratio--an indicator of alcoholic liver disease." Dig Dis Sci 24(11): 835-838.

Dominguez, M., et al. (2008). "A new scoring system for prognostic stratification of patients with alcoholic hepatitis." Am J Gastroenterol 103(11): 2747-2756.

Forrest, E. H., et al. (2005). "Analysis of factors predictive of mortality in alcoholic hepatitis and derivation and validation of the Glasgow alcoholic hepatitis score." Gut 54(8): 1174-1179.

Forrest, E. H. and D. Gleeson (2012). "Is a liver biopsy necessary in alcoholic hepatitis?" J Hepatol 56(6): 1427-1428; author reply 1428-1429.

Fujimoto, M., et al. (2000). "Plasma endotoxin and serum cytokine levels in patients with alcoholic hepatitis: relation to severity of liver disturbance." Alcohol Clin Exp Res 24(4 Suppl): 48S-54S.

Kamath, P. S., et al. (2001). "A model to predict survival in patients with end-stage liver disease." Hepatology 33(2): 464-470.

Louvet, A., et al. (2007). "The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids." Hepatology 45(6): 1348-1354.

Lucey, M. R., et al. (2009). "Alcoholic hepatitis." N Engl J Med 360(26): 2758-2769.

Maddrey, W. C., et al. (1978). "Corticosteroid therapy of alcoholic hepatitis." Gastroenterology 75(2): 193-199.

Marra, F. and F. Tacke (2014). "Roles for chemokines in liver disease." Gastroenterology 147(3): 577-594 e571.

Michelena, J., et al. (2015). "Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis." Hepatology 62(3): 762-772.

Mookerjee, R. P., et al. (2011). "The role of liver biopsy in the diagnosis and prognosis of patients with acute deterioration of alcoholic cirrhosis." J Hepatol 55(5): 1103-1111.

Sheela, H., et al. (2005). "Liver biopsy: evolving role in the new millennium." J Clin Gastroenterol 39(7): 603-610.

Srikureja, W., et al. (2005). "MELD score is a better prognostic model than Child-Turcotte-Pugh score or Discriminant Function score in patients with alcoholic hepatitis." J Hepatol 42(5): 700-706.

Uchida, T., et al. (1984). "Giant mitochondria in the alcoholic liver diseases--their identification, frequency and pathologic significance." Liver 4(1): 29-38.

Yeluru, A., et al. (2016). "Alcoholic Hepatitis: Risk Factors, Pathogenesis, and Approach to Treatment." Alcohol Clin Exp Res 40(2): 246-255.

VITAE

Apurva Yeluru was born in India but now calls San Antonio, TX her home. She did her undergraduate studies at Johns Hopkins University, where she majored in Biomedical Engineering. She went to medical school at UT Southwestern Medical Center in Dallas, TX. During medical school, she spent a year doing clinical research on alcoholic hepatitis. She graduated from medical school in 2016, and is going to Stanford University for her Internal Medicine residency. She plans to do a fellowship in Gastroenterology and Hepatology. In her free time, Apurva enjoys traveling and photography.

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