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Stratification of Risk of Death in Severe Acute Alcoholic Hepatitis Using a Panel of Adipokines and Cytokines

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Abstract

Background: Dysregulated adipose tissue metabolism has been implicated in the pathogenesis of alcoholic liver disease in murine models. We aimed to characterize serum markers of adipose tissue metabolism and inflammation in patients with severe acute alcoholic hepatitis (AAH) and determine their utility to predict survival in severe AAH.

Methods: A prospective, case control study design was used. Seventy-six patients hospitalized with severe AAH and twenty-five ambulatory patients with alcoholic cirrhosis as controls were included. Serum samples were collected for biochemical analyses. Patients were followed for 180 days after enrollment to determine survival.

Results: AAH patients exhibited higher serum glycerol and free fatty acid levels, suggesting enhanced adipose tissue triglyceride hydrolysis. Patients with AAH demonstrated a distinct serum lipidomic profile compared with alcoholic cirrhosis but not in systemic and adipose-specific insulin resistance. AAH patients had higher serum resistin and plasmin activation inhibitor-1 levels, while serum leptin was decreased. Serum levels of the proinflammatory cytokines tumor necrosis factor α (TNF- α), interleukin (IL)-6, IL-8 and IL-15 were significantly higher in AAH patients. Only 53% of AAH patients survived 180 days after admission, while all cirrhotic patients were alive at the end of the study period. Among patients with severe AAH, white blood cell count, hemoglobin, resistin, IL-6, and TNF- α were associated with 180-day survival, and all five markers demonstrated accuracy by area under receiver-operator curve (AUC) analysis. Serum IL-6 levels 38.66 pg/ml most precisely identified deaths in severe AAH. Patients with IL-6 38.66 pg/ml had significantly decreased mean survival compared to those with lower levels.

Conclusions: AAH patients demonstrate evidence of increased adipose tissue lipolysis and altered serum lipidomic profile compared with alcoholic cirrhosis patients. IL-6 may be a useful biomarker to risk-stratify severe AAH patients at the highest risk of mortality.

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Keywords

Acute alcoholic hepatitis; biomarker; adipose tissue metabolism; IL-6; serum lipidomic analysis

Introduction

Acute alcoholic hepatitis (AAH) is a multisystem condition characterized by jaundice, hepatomegaly, hepatic failure and features of the systemic inflammatory response syndrome (SIRS). While the exact incidence is unknown, a prevalence of up to 20% has been reported among individuals with documented alcoholism (Lucey et al., 2009, Naveau et al., 1997). Although multiple scoring systems have been developed to predict mortality in AAH, Maddrey discriminant function (MDF) is most commonly used (Maddrey et al., 1978). An MDF ≥ 32 with or without hepatic encephalopathy is associated with 30-50% one-month mortality (Mathurin et al., 1996) and necessitates pharmacologic therapy with either corticosteroids or pentoxifylline (O'Shea et al., 2010). Despite treatment, one month mortality is as high as 20% with long-term mortality over 39% (Rambaldi et al., 2008, Fleming et al., 2010), suggesting that alternative measures are required to predict survival in high-risk patients.

Cardinal histologic findings of AAH include ballooning hepatocytes, Mallory-Denk bodies (eosinophilic hepatocellular inclusion bodies) and neutrophilic infiltration in the setting of macrovesicular steatosis, or hepatocellular lipid droplet formation (European Association for the Study of, 2012). Adipose tissue likely represents a major source of intrahepatic lipids in alcoholic liver disease, as dysregulated adipose tissue metabolism and associated inflammation have been described in animal models and human studies of alcoholism. In noncirrhotic alcoholic subjects, decreased adipose mass is associated with increased steatosis (Addolorato et al., 1997, Addolorato et al., 1998), and in rodent models, ethanol exposure simultaneously enhanced hepatic fat accumulation while reducing peripheral adipose mass (Kang et al., 2007a). Subsequent murine studies demonstrated that ethanol enhances adipose tissue lipolysis by inhibiting insulin-mediated negative regulation of triglyceride hydrolysis (Zhong et al., 2012). Multiple cytokines elaborated in AAH including tumor necrosis factor- α (TNF- α), interleukins-6 (IL-6) and -8 (IL-8) are also upregulated in adipose fat after ethanol exposure (Naveau et al., 2010, Kang et al., 2007b). Furthermore, adipose-derived inflammatory factors, or adipokines, contribute to hepatic inflammation (Bertolani et al., 2006, Marra and Bertolani, 2009, Purohit et al., 2009, Rogers et al., 2008).

As adipose tissue appears to play a role in the pathogenesis of alcoholic liver disease, we hypothesize that dysregulated fat metabolism and associated inflammation may add prognostic value to traditional measures of mortality risk among patients with severe AAH. We prospectively assessed adipose tissue lipolysis, insulin resistance, and serum adipocytokine levels in hospitalized patients with severe AAH and in stable cirrhotic subjects. We then considered correlations between these features and the Model for End-Stage Liver Diseases (MELD), a commonly used prediction tool (Sheth et al., 2002, Dunn et al., 2005). Finally, we determined the prognostic ability of markers of adipose tissue dysregulation to predict survival in severe AAH.

Methods

Study Design and Participants

This prospective case-control study was approved by the institutional review board (IRB) at the University of Pittsburgh. Patients with a diagnosis of severe AAH (Maddrey Discriminant Function (MDF) score ≥ 32) were enrolled at the University of Pittsburgh Medical Center Presbyterian Hospital in Pittsburgh, Pennsylvania from October 2010 to April 2012. Informed consent was obtained prior to inclusion in the study from either the subject or a designated representative. Inclusion criteria included: age ≥ 18 years, history of chronic, heavy alcohol abuse, total bilirubin level of ≥ 3 mg/dL with aspartate aminotransferase (AST) greater than alanine aminotransferase (ALT) activity and both levels < 500 U/L, and the presence of either hepatomegaly or steatosis by ultrasound or computed tomography (CT) imaging. Subjects who were abstinent from alcohol for at least 6 weeks prior to admission were excluded. Three patients in the cohort underwent transjugular liver biopsy and findings were consistent with alcoholic hepatitis. All patients with severe AAH received supportive therapy including nutrition and vitamin supplementation. Medical treatment with corticosteroids and/or pentoxifylline was initiated at the discretion of the treating hepatologist.

As over 70% of patients with AAH have histologic evidence of cirrhosis (Hamid and Forrest, 2011), a control group of cirrhotic subjects was generated from the IRB-approved University of Pittsburgh Center for Liver Diseases Research Registry. The registry consists of clinically stable cirrhotic individuals presenting for outpatient hepatology care. All registry participants previously consented to inclusion in the registry and provided serum samples for research purposes. 25 patients with a diagnosis of alcoholic cirrhosis (ICD-9 code 571.2) with or without chronic viral hepatitis were identified from the registry and included in the control group. All patient charts were reviewed to confirm the absence of alcoholic hepatitis. We used a second control group of 15 age-, gender- and race-matched patients from the North American Pancreatitis Study (NAPS2), without ethanol use, liver disease, or pancreatic disease to measure levels of free fatty acids and glycerol. All samples were otherwise de-identified (Whitcomb et al., 2008).

Clinical and Demographic Evaluation

Patient age, gender, and ethnicity were recorded, and six-month patient survival was determined using the Social Security Death Index. Body mass index (BMI) was calculated from height and body mass measurements using the following formula (Garrow and Webster, 1985): $BMI = \text{mass (in kg)} / (\text{height in m})^2$. All patients underwent routine clinical laboratory measurements including serum AST, ALT, total bilirubin, alkaline phosphatase, prothrombin time (PT), international normalized ratio (INR), albumin, total protein, glucose, blood urea nitrogen, and creatinine at their index clinical encounter. Maddrey Discriminant Function (MDF) was determined using the following formula (Maddrey et al., 1978): $MDF = [PT \text{ (sec)} - \text{control PT (sec)}] \times 4.6 + \text{total bilirubin (mg/dl)}$. A laboratory control PT of 12.5 seconds is routinely used at our institution. The MELD score was calculated from the following equation (Dunn et al., 2005): $MELD = (0.957 \times \log [\text{creatinine (mg/dl)}]) + 0.378 \times \log[\text{total bilirubin(mg/dl)}] + 1.120 \times \log (\text{INR}) + 0.643$. Patients who received at least

two renal replacement treatments within one week of enrollment were assigned a creatinine value of 4.0 mg/dl when calculating MELD. On admission, complete blood cell counts (CBC) were measured as part of routine clinical care.

Serum Cytokine and Adipokine Measurement

Twenty ml of blood was collected into EDTA-containing tubes for serological and metabolic analysis. Samples were collected in the early morning between 4-6 AM within 24 hours of admission. Serum was obtained by centrifugation and stored at -80°C until testing. Levels of cytokines (TNF- α , IL-6, IL-8, and IL-15) and adipokines (adiponectin, adipisin, resistin, leptin, and plasminogen activation inhibitor-1 (PAI-1)) were measured on a Luminex platform at the University of Pittsburgh Genomics and Proteomics Core facility (Pittsburgh, PA).

Serum Fatty Acid and Glycerol Analysis

To assess serum glycerol and free fatty acid (FFA) concentrations, each sample underwent extraction with isopropanol-heptane-hydrochloric acid (1M) (40:10:1, v/v) by vortexing for 25-30 min, followed by incubation at room temperature for 10 min. Heptane (4.0 ml) and water (2.0 ml) were added and the tubes were thoroughly vortexed for 5 min. After centrifugation at 1000 g for 10 min at 4°C , the upper phase (heptane) was transferred into 13×100 mm screw top tubes and dried in a SpeedVac centrifugal concentrator (Thermo Scientific, Waltham, MA). Free fatty acids were derivatized with dimethylamine using the Deoxo-Fluor reagent (Sigma Aldrich, St. Louis, MO) (Kangani et al., 2008). Free fatty acid concentrations were measured by gas chromatography separation with flame ionization detection using heptanoic acid as an internal standard. This derivatization method is very mild and efficient, and is selective only towards FFAs so that no separation from a total lipid extract is required (Kangani et al., 2008).

Serum Insulin and Insulin Resistance Determination

Serum insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) for human insulin according to the manufacturer's instructions (EMD Millipore, Billerica, MA). Whole-body insulin resistance was determined using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), a well-validated estimation of insulin resistance as determined by euglycemic-hyperinsulinemic clamp experiments (Hermans et al., 1999, Wallace et al., 2004): $\text{HOMA-IR} = \text{Glucose (mg/dl)} \times \text{Insulin (mU/L)} / 405$. Adipose-tissue specific insulin resistance was calculated using the ADIPO-IR index, which was first introduced in therapeutic studies of nonalcoholic fatty liver disease (Gastaldelli et al., 2009, Bell et al., 2012): $\text{ADIPO-IR} = \text{Free fatty acids (}\mu\text{M)} \times \text{Insulin (mU/L)}$.

Statistical Analysis

Statistical analysis was performed with Stata version 11 (StataCorp, College Station, TX) and SigmaPlot version 12 (Systat Software, San Jose, CA). Clinical and demographic characteristics were reported with absolute frequencies, percentages, medians, and interquartile ranges (IQR). Comparisons between groups were performed with the χ^2 test for categorical variables (or Fischer's exact test when expected values were < 5), while the

Mann Whitney *U* test was used for continuous variables. Correlation between variables was determined using Spearman's rank correlation coefficient (ρ) method. Univariate Cox proportional hazards analysis was used to determine predictors of 6-month mortality. No patients were lost to follow-up, and subjects who survived for the entire follow-up period were censored at 180 days. Area under receiver-operator curve (AUROC) analysis was performed to determine the accuracy of clinical variables for mortality prediction, and optimal thresholds for candidate variables were estimated from ROC curves using Youden's Index Method (Perkins and Schisterman, 2005). Survival times were analyzed with Kaplan Meier methods, and survival curves were compared with the log-rank method. A *p* value (two-tailed) less than or equal to 0.05 was considered statistically significant.

Results

Clinical and demographic features of patients

Clinical and demographic characteristics are summarized in Table 1. The control population consisted of twenty-five clinically stable cirrhotic patients, while the AAH cohort was comprised of 76 subjects. The AAH population was slightly older, but gender and ethnic distribution were similar in the two groups. Both groups had similar incidence of diabetes, and both populations were overweight by BMI. AAH patients exhibited higher MELD scores, transaminase levels and WBC counts with decreased hemoglobin and albumin. While all cirrhotic patients were alive at the end of the study period, only 53% of AAH patients survived beyond 180 days.

AAH patients exhibit evidence of increased lipolysis in the absence of insulin resistance

To assess adipose tissue lipolysis, serum FFA and glycerol were measured. Patients with AAH had significantly elevated FFA and glycerol levels compared to cirrhotic controls despite similar BMI. Furthermore, serum glycerol levels increased by nearly five-fold in AAH patients (Figure 1A). Serum lipidomic analysis was then performed to determine whether the increase in FFA was due to a rise in specific FFA species or rather represented a global change in FFA levels. Serum palmitate (C16:0), palmitoleate (C16:1), and oleate (C18:1) were significantly higher in AAH patients, while other FFA moieties tested were similar in both groups (Figure 1B); the monounsaturated: saturated FFA ratio was increased in severe AAH (114.7 ± 26.9 versus $201.6 \pm 18.0 \mu\text{M}$, $p = 0.012$). Thus, AAH is associated with evidence of increased lipolysis with enhanced production of specific FFA species.

Adipose tissue lipolysis is inhibited by insulin, and murine studies indicate that ethanol-induced lipolysis may be mediated by adipose tissue insulin resistance (IR) (Kang et al., 2007a, Zhong et al., 2012). To determine the presence of IR as a contributor to increased adipose tissue lipolysis, serum glucose and insulin levels were measured. Although patients with AAH exhibited increased serum glucose levels compared to cirrhotic controls, insulin levels were similar in both groups. In addition, markers of systemic (HOMA-IR) and adipose tissue-specific (ADIPO-IR) IR were unchanged (Figure 1A), suggesting that enhanced lipolysis occurs independently of IR in AAH.

Serum adipokine and cytokine levels are altered in AAH patients

Adipose tissue-derived cytokines, or adipokines, have been shown to modulate hepatic metabolism and development of steatosis in the setting of chronic ethanol ingestion (Endo et al., 2007, Xu et al., 2003). Furthermore, inflammatory cytokines associated with alcoholic liver disease also stimulate adipose tissue lipolysis (Cawthorn and Sethi, 2008, Trujillo et al., 2004, Ajuwon and Spurlock, 2004). We thus measured serum levels of major adipokines and cytokines implicated in alcoholic liver disease. Among adipokines, resistin was markedly increased in AAH patients compared to cirrhotic controls, and PAI-1 expression was mildly enhanced in subjects with AAH; serum leptin levels were decreased in AAH patients, while adiponectin and adipsin levels were similar between groups (Figure 2A). Among inflammatory mediators assayed, the proinflammatory cytokines TNF α , IL-6, IL-8, and IL-15 were significantly increased in patients with AAH when compared to cirrhotic subjects (Figure 2B). These findings demonstrate that adipose tissue inflammation accompanies enhanced lipolysis in severe AAH.

To determine the clinical significance of adipokine and cytokine profiles in AAH, we assessed correlations with the MELD score, a commonly used measure of disease severity in both cirrhosis and alcoholic hepatitis (Dunn et al., 2005). In patients with severe AAH, serum levels of TNF α , adipsin, and resistin were positively correlated with MELD (Figure 3), while IL-6, IL-8, IL-15, PAI-1, adiponectin, and leptin failed to demonstrate significant correlations. In addition, white blood cell count was significantly correlated with MELD score. As adipocytokine expression profiles were discordant with MELD correlations, it is possible that the MELD score loses discriminative power in patients with MDF. Instead, markers of adipose tissue dysregulation may distinguish patients at increased risk of mortality in severe AAH.

Serum resistin, TNF α , IL-6, white blood cell count, and hemoglobin levels identify long-term survivors in severe AAH

In patients with severe AAH, The mean overall survival time was 118.5 days (95% C.I. 101.2-135.9 days) (Figure 4). No stable cirrhotic patients died during the study period. Mortality was not related to therapeutic decisions, as both survivors and non-survivors were similarly exposed to corticosteroids (38.1% of survivors versus 45.5% of non-survivors, $p = \text{N.S.}$), pentoxifylline (61.9% versus 60.2%, $p = \text{N.S.}$), both agents (7.1% versus 18.1%, $p = \text{N.S.}$), or neither drug (7.1% versus 12.1%, $p = \text{N.S.}$).

Given the poor survival of patients with severe AAH, univariate Cox proportional hazards analysis was used to determine clinical, demographic, metabolic and serologic factors associated with 6 month mortality. Among the variables studied, WBC count, hemoglobin, resistin, TNF α , IL-6, IL-8 and IL-15 were significantly associated with survival, while MELD and MDF were not. (Table 2) To further assess the prognostic utility of these factors, area under receiver-operator curve (AUROC) analysis was performed. While IL-8 and IL-15 poorly discriminated mortality risk [IL-8 AUROC 0.610 (0.445-0.776), $p = 0.190$; IL-15 AUROC 0.647 (95% C.I. 0.479-0.814), $p = 0.089$], WBC count, hemoglobin, resistin, TNF α and IL-6 accurately characterized survivors and non-survivors, as determined by AUROC 0.7. (Table 3 and Figure 4) Optimal threshold values for these parameters were then

determined from ROC curves using Youden's Index method (Schisterman et al., 2005). Log-rank analysis of Kaplan-Meier survival curves stratified by cutoff values for each variable was then performed, and serum IL-6 levels ≥ 38.66 pg/ml most precisely identified deaths in severe AAH (Table 4). Patients with IL-6 ≥ 38.66 pg/ml had significantly decreased mean survival (42.9 days, 95% C.I. 11.11-74.65 days) compared to those with low levels (158.8 days, 95% C.I. 140-170.6 days, $p < 0.001$) (Figure 5). Overall these findings identify a specific set of markers of inflammation and adipose tissue dysregulation that accurately predict 180 day survival in patients with severe AAH.

Discussion

In this prospective case-control study, we addressed the role of adipose tissue dysfunction and associated inflammation in severe AAH. Three novel observations were made. First, patients with severe AAH exhibit enhanced levels of serum glycerol and FFA due to increased lipolysis of specific lipid moieties. Second, adipose tissue inflammation occurs in severe AAH and is characterized by specific patterns of adipokine and cytokine expression; these profiles conflicted with correlations to MELD, thus suggesting that MELD may not accurately describe disease severity in patients with elevated MDF. Third, we characterized five parameters that accurately predict survival in severe AAH. Among these, elevated serum IL-6 levels most precisely identified high-risk patients.

Recent evidence suggest that fat translocation from adipose compartments contributes to steatosis and associated hepatic inflammation in alcoholic liver disease (Zhong et al., 2012, Kang et al., 2007a). In rodent models, chronic ethanol exposure was associated with decreased adipose fat mass and enhanced ex-vivo adipose triglyceride hydrolysis; furthermore, activation of adipose triglyceride lipase and hormone-sensitive lipase (key enzymes for lipolysis) was increased in alcohol-fed mice (Zhong et al., 2012). Using deuterium-labeling experiments, investigators demonstrated hepatic uptake of adipose-derived FFA in ethanol-fed mice (Wei et al., 2013). Consistent with these findings, patients with severe AAH in our study exhibited evidence of enhanced lipolysis when compared to stable cirrhotic subjects; in particular, serum concentrations of specific saturated and monounsaturated free fatty acids were markedly increased. Previous animal models demonstrated that ethanol-induced adipose tissue lipolysis is mediated by insulin resistance, as ethanol exposure reduced insulin-mediated negative regulation of lipolysis in rodents (Kang et al., 2007a). In the current study, however, both global and adipose-specific IR was similar between experimental groups.

Multiple adipokines, including resistin, were significantly elevated in patients with severe AAH. In mice, resistin expression is restricted to adipocytes (Steppan et al., 2001) while in humans, it localized to monocytes and macrophages (Savage et al., 2001). In studies of mice expressing humanized resistin, hepatic steatosis was associated with increased macrophage translocation to adipose tissue, which resulted in increased adipose tissue resistin content, upregulated adipose expression of mRNA for inflammatory cytokines, and enhanced adipose lipolysis (Qatanani et al., 2009). Similarly, in patients with alcoholic hepatitis, intrahepatic resistin was localized to macrophages, hepatic stellate cells, and Kupffer cells (Bertolani et al., 2006). Collectively, these observations suggest that ethanol-induced

inflammation may drive lipolysis through resistin-mediated upregulation of proinflammatory cytokines, and in the current study TNF α , IL-6, IL-8, and IL-15 were increased in patients with severe AAH.

As only half of all patients with severe AAH survived to 6 months, we sought to determine clinical, metabolic, and serologic markers to predict mortality risk. Using Cox proportional hazards methods and AUROC analysis, we characterized five parameters – WBC, hemoglobin, TNF α , IL-6, and resistin – that were significantly associated with 180 day mortality; interestingly, neither MDF nor MELD were associated with survival. Evaluation of AUROC curves demonstrated that serum IL-6 levels best predicted survival in this cohort, and patients with IL-6 ≥ 38.66 pg/ml had a 3-fold decrease in survival time compared to those with lower values. Serum IL-6 elevation has been previously observed in AAH (Sheron et al., 1991, Hill et al., 1992, Fujimoto et al., 2000, Mookerjee et al., 2003), but reported discrimination levels are much higher than those presented here. This may be explained by a number of factors, such as the use of different IL-6 measurement platforms and the inclusion of both severe and milder cases of AAH (i.e. patients with MDF < 32) in previous work. While MDF, MELD, and other continuous scoring systems identify patients with severe AAH, a significant proportion do not survive despite pharmacologic therapy, thus highlighting the need for further characterization of mortality risk. In the current study, we have identified IL-6 as a robust predictor of survival among patients treated for severe AAH. We thus propose serum IL-6 measurement as a simple, noninvasive test to enhance risk prediction among patients with severe AAH, and future studies are planned to validate this finding in a separate prospective-followed cohort.

A few limitations are noted. First, the diagnosis of AAH was made on clinical grounds, and only three patients underwent liver biopsy. While prior studies have confirmed the utility of liver biopsy in AAH (Mookerjee et al., 2011), a recent meta-analysis of therapeutic trials requiring biopsy-proven AAH for inclusion demonstrated 96% histologic confirmation in patients with serum bilirubin > 80 μ mol/L (4.7 mg/dl) (Forrest and Gleeson, 2012). Next, our analysis was not sufficiently powered to detect acute kidney injury and hepatorenal syndrome, and future, larger investigations are planned to detect the effect of lipid metabolism on renal dysfunction. As this was an observational study, patient management was determined by attending hepatologists, and no *a priori* treatment algorithm was assigned. However, treatment patterns were similar between survivors and non-survivors. Finally, serum cytokines and adipokines were assessed once upon enrollment, and future studies are needed to determine whether fluctuations in inflammation profiles predict survival. Nonetheless, this study provides a detailed analysis of adipose tissue metabolism and associated inflammation in prospectively studied, well-matched cohorts for whom extensive clinical data were available.

In conclusion, our findings demonstrate that dysregulation of adipose tissue metabolism and associated inflammation are implicated in the pathogenesis of AAH. Furthermore, markers of adipose tissue dysfunction provide prognostic value in patients with severe AAH and help stratify patients at the highest risk of mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Serum glycerol, nonesterified fatty acids (NEFA), glucose, insulin, homeostasis model assessment-insulin resistance (HOMA-IR) and adipose tissue insulin resistance (ADIPO-IR) levels in patients with severe acute alcoholic hepatitis (AAH) and stable alcoholic cirrhosis (cirrhosis). (B) Distribution of serum fatty acid moieties in patients with AAH and cirrhosis.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2.

(A) Serum adipokine levels and (B) serum cytokine levels in patients with severe acute alcoholic hepatitis (AAH) and stable alcoholic cirrhosis (cirrhosis). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3. Statistically significant correlations for model of end-stage liver disease (MELD) with associated scatter plots. TNF α , tumor necrosis factor alpha; WBC, white blood cell count.

Figure 4.

Receiver-operator characteristic curves for mortality at 180 days. A, area under receiver-operator curve; Hgb, hemoglobin; IL-6, interleukin-6; TNF α , tumor necrosis factor alpha; WBC, white blood cell count.

Figure 5.

(A) Overall 180-day survival curve for patients with severe acute alcoholic hepatitis. (B) Survival curves of severe alcoholic hepatitis patients stratified by interleukin-6 cutoff of 38.66 pg/ml. Survival curves were compared using log-rank methods. High IL-6, interleukin-6 level ≥ 38.66 pg/ml; Low IL-6, interleukin-6 level < 38.66 pg/ml.

Table 1

Clinical and Demographic Characteristics of Patients

Characteristics	AAH (N=76)	Cirrhosis (N=25)	<i>P</i>
Age, yrs	49 (39-53)	55 (46-57)	0.007
Male, n (%)	55 (72%)	18 (72%)	NS
Ethnicity, n (%)			
Caucasian	72 (95%)	24 (96%)	NS
Black	3 (4%)	1 (4%)	NS
Hispanic	1 (1%)	0 (0%)	NS
Other Liver Diseases, n (%)	8 (10.5%)	11 (44%)	0.001
Hepatitis C	6 (7.9%)	10 (40%)	0.001
Hepatitis B	1 (1.3%)	1 (4%)	NS
Other	1 (1.3%)	0 (0%)	NS
Body Mass Index, kg/cm ²	27.9 (24.5-33.6)	27.6 (25.3-30.1)	NS
Diabetes, n (%)	8 (11%)	3 (12%)	NS
Maddrey's Discriminant Function	52.5 (38.2-68.8)	N/A	N/A
MELD	26 (23-31)	11 (9-14)	< 0.001
Albumin, g/L	2.1 (1.8-2.3)	3.2 (2.9-4.1)	< 0.001
Bilirubin, mg/dL	15.2 (9.6-25.6)	1.3 (1.0-2.1)	< 0.001
AST, IU/L	138 (96-203)	26 (20-51)	< 0.001
ALT, IU/L	47 (37-64)	43 (32-63)	0.003
INR	2.0 (1.7-2.4)	1.3 (1.2-1.5)	< 0.001
Creatinine, mg/dL	1.0 (0.8-1.6)	0.8 (0.8-1.1)	NS
WBC count, ×10 ³ /ml	11.1 (7.7-14.6)	5.6 (4.2-6.8)	< 0.001
Hemoglobin, gm/dl	10.6 (9.4-11.8)	13.6 (12.7-14.9)	< 0.001
Platelet count, ×10 ³ /ml	114 (75-170)	89 (67-140)	NS
30-day survival, n (%)	57 (74%)	25 (100%)	< 0.001
90-day survival, n (%)	47 (61%)	25 (100%)	< 0.001
180-day survival, n (%)	41 (53%)	25 (100%)	< 0.001

Table 1: Baseline characteristics of patients. All values are expressed as medians and IQRs unless otherwise specified. Continuous variables were compared with Mann-Whitney *U* test. Categorical variables were compared with Fisher's exact test. N/A: not applicable; NS: not significant (*p* > 0.05).

Table 2

Predictors of 180 day survival by univariate Cox proportional hazards analysis

Characteristics	Hazard Ratio	95% C.I.	P Value
Age	1.015	0.976 – 1.057	0.453
Female gender	1.131	0.475 – 2.692	0.781
African American race	1.972	0.461 – 8.426	0.359
BMI	1.004	0.947 – 1.065	0.887
Glucose	1.008	0.997 – 1.019	0.145
Insulin	0.987	0.936 – 1.040	0.621
Total FFA	1.001	1.000 – 1.002	0.146
HOMA-IR	0.972	0.823 – 1.149	0.740
ADIPO-IR	1.000	0.999 – 1.000	0.394
MELD	1.054	0.974 – 1.141	0.189
Maddrey's Discriminant Function	1.008	0.996 – 1.020	0.190
AST	1.000	0.998 – 1.003	0.572
ALT	1.004	0.994 – 1.014	0.458
Bilirubin	1.014	0.974 – 1.056	0.502
Alkaline phosphatase	1.000	0.997 – 1.003	0.861
Albumin	1.256	0.571 – 2.757	0.571
Creatinine	1.377	0.908 – 2.088	0.132
White blood cell count	1.109	1.043 – 1.179	0.001
Hemoglobin	0.814	0.689 – 0.962	0.016
Platelet count	1.000	0.997 – 1.004	0.850
INR	1.206	0.755 – 1.928	0.432
Adiponectin	1.075	0.904 – 1.081	0.802
Leptin	0.970	0.920 – 1.023	0.268
Adipsin	1.075	0.863 – 1.339	0.522
PAI-1	1.007	0.999 – 1.015	0.106
Resistin	1.044	1.025 – 1.062	< 0.001
TNF-α	1.036	1.010 – 1.062	0.005
IL-6	1.010	1.005 – 1.014	< 0.001
IL-8	1.001	1.000 – 1.002	0.009
IL-15	1.066	1.003 – 1.131	0.038

Table 2: Univariate predictors of 180 day survival by Cox proportional hazards analysis. Statistically significant characteristic characteristics are in bold ($p < 0.05$). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ADIPO-IR, adipose tissue insulin resistance; FFA, free fatty acids; HOMA-IR, homeostasis model of assessment-insulin resistance; IL-6, interleukin-6; IL-8, interleukin-8; IL-15, interleukin-15; INR, international normalized ratio; MDF, Maddrey Discriminant Function; MELD, model for end stage liver disease; PAI-1, platelet activation inhibitor-1; TNF- α , tumor necrosis factor-alpha; WBC, white blood cell count.

Table 3

Receiver-operator characteristic analysis of prognostic variables for mortality at 180 days in severe alcoholic hepatitis.

Characteristics	AUC (95% C.I.)	<i>P</i>	Cutoff	Sensitivity	Specificity
Resistin	0.75 (0.61 – 0.89)	0.003	13.15 ng/ml	0.64	0.70
Interleukin-6	0.80 (0.65 – 0.94)	< 0.001	38.66 pg/ml	0.96	0.74
Tumor Necrosis Factor-α	0.74 (0.60 – 0.88)	0.004	10.07 pg/ml	0.64	0.78
	0.73 (0.59 – 0.86)	0.004	10.75 10^{-3} cc	0.59	0.77
WBC count	0.70 (0.56 – 0.83)	0.01	11.50 gm/dl	0.41	0.92
Hemoglobin					

Table 3: Assessment of prognostic factors for 180 day mortality prediction in severe alcoholic hepatitis. Variables with statistically significance by univariate logistic regression were studied using receiver-operator characteristic analysis. Prediction accuracy was determined by measuring the area under each curve. Optimal cutoff values for each variable with associated sensitivity and specificity were determined using Youden's Index Method. AUC, area under receiver operator curve.

Table 4

Survival analysis of patients with severe alcoholic hepatitis, stratified by optimal thresholds of prognostic variables.

Characteristics	Cutoff Value	Mean Survival (days)*	95% C.I. (days)	P **
Resistin	13.15 ng/ml	82.44	49.62-115.16	0.009
Interleukin-6	38.66 pg/ml	42.88	11.11-74.65	< 0.001
Tumor Necrosis Factor-α	10.07 pg/ml	84.78	53.09-116.47	0.006
	10.75 10^{-3} cc	91.16	62.46-119.86	0.024
WBC count	11.50 gm/dl	98.73	73.51-123.96	0.018
Hemoglobin				

Table 4: 180 day survival analysis of patients with severe alcoholic hepatitis stratified by optimal thresholds of prognostic variables.

* Mean survival of patients with levels equal to or greater than the optimal cutoff (in the case of hemoglobin, mean survival of patients with hemoglobin levels below the optimal cutoff value are reported).

** P values are reported for survival curves stratified by cutoff values for each prognostic variable using log-rank analysis.