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Research Article

Serum Cytokine and Inflammatory Marker Profiles as Prognostic Indicators in Alcoholic Liver Disease

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ABSTRACT

Background: Alcoholic liver disease (ALD) is a leading cause of chronic liver disease worldwide, characterized by a spectrum of pathologies driven by chronic inflammation. While scoring systems like the Model for End-Stage Liver Disease (MELD) are used for prognosis, they primarily reflect organ dysfunction rather than the underlying inflammatory activity. There is a need for biomarkers that can better stratify patient risk.

Methods: We conducted a prospective cohort study involving 150 patients with a confirmed diagnosis of ALD and 50 healthy controls. ALD patients were stratified into three groups based on MELD score: Low Severity (MELD < 15, n=50), Moderate Severity (MELD 15–25, n=50), and High Severity (MELD > 25, n=50). Serum levels of TNF-α, IL-6, IL-10, hs-CRP, and ESR were measured at baseline using standardized assays. All patients were followed for 90 days to assess all-cause mortality.

Key Findings: Serum levels of pro-inflammatory markers were significantly elevated in ALD patients compared to controls ($p<0.001$) and increased in a severity-dependent manner. Mean IL-6 levels were 8.2 ± 2.1 pg/mL in controls, 45.6 ± 11.2 pg/mL in the low-severity group, 112.8 ± 25.4 pg/mL in the moderate-severity group, and 245.3 ± 58.7 pg/mL in the high-severity group ($p<0.001$). Similarly, TNF-α levels were significantly higher in the high-severity group (85.2 ± 15.1 pg/mL) compared to the low-severity group (30.5 ± 8.2 pg/mL) and controls (5.1 ± 1.8 pg/mL) ($p<0.001$). Over the 90-day follow-up, 28 (18.7%) ALD patients died. In a multivariate Cox proportional hazards model adjusted for age, sex, and MELD score, both baseline log-transformed IL-6 (Hazard Ratio [HR]: 2.85; 95% CI: 1.52–5.34; $p=0.001$) and log-transformed TNF-α (HR: 2.10; 95% CI: 1.15–3.84; $p=0.015$) remained independent predictors of 90-day mortality.

Conclusion: Serum concentrations of TNF-α and IL-6 are strongly associated with the severity of ALD and serve as independent prognostic indicators of short-term mortality. These biomarkers may supplement the MELD score to improve risk stratification and guide clinical management in patients with ALD.

Keywords: *Alcoholic Liver Disease, Cytokines, Inflammation, Prognosis, TNF-α, IL-6, MELD Score.*

INTRODUCTION

Alcoholic liver disease (ALD) represents a major global health burden, encompassing a histological spectrum from simple steatosis to steatohepatitis, fibrosis, and ultimately cirrhosis and hepatocellular carcinoma **[1]**. Chronic excessive alcohol consumption is the primary etiological factor, leading to significant morbidity and mortality worldwide. The pathogenesis of ALD is complex and multifactorial, but sterile inflammation is now recognized as a central driver of disease progression **[2]**.

The metabolism of ethanol in the liver generates reactive oxygen species (ROS) and acetaldehyde, which promote hepatocyte injury and oxidative stress. Concurrently, chronic alcohol use increases intestinal permeability, leading to the

translocation of gut-derived microbial products, such as lipopolysaccharide (LPS), into the portal circulation **[3]**. LPS activates Kupffer cells, the resident macrophages of the liver, via Toll-like receptor 4 (TLR4) signaling. This activation triggers a robust inflammatory cascade characterized by the production of pro-inflammatory cytokines, including Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) **[4]**. TNF-α is a pivotal cytokine in ALD, promoting hepatocyte apoptosis, insulin resistance, and the expression of other inflammatory mediators. IL-6 plays a dual role, contributing to inflammation and liver injury while also being involved in the acute-phase response and hepatocyte regeneration **[5]**. This pro-inflammatory state is counterbalanced by anti-inflammatory cytokines like Interleukin-10 (IL-10), but in severe ALD, this response is often insufficient to quell the inflammatory damage **[6]**.

Accurate prognostication in ALD is critical for clinical decision-making, including prioritization for liver transplantation. The Model for End-Stage Liver Disease (MELD) score, based on serum bilirubin, creatinine, and the international normalized ratio (INR), is the most widely used tool for assessing 3-month mortality risk in patients with end-stage liver disease **[7]**. However, the MELD score primarily reflects established hepatic and renal dysfunction and may not fully capture the dynamic and systemic inflammatory state that drives mortality, particularly in acute-on-chronic liver failure. Several studies have highlighted the limitations of the MELD score and explored the addition of inflammatory markers, such as C-reactive protein (CRP), to improve its predictive accuracy **[8]**.

Recent research has focused on the direct role of cytokines as biomarkers. Studies have shown elevated levels of IL-6 and IL-8 in patients with severe alcoholic hepatitis, correlating with poor outcomes **[9]**. Similarly, high TNF-α levels have been linked to increased mortality risk **[10]**. Despite these findings, a research gap remains in comprehensively profiling multiple key cytokines (both pro- and anti-inflammatory) alongside conventional markers in a well-stratified ALD cohort and rigorously testing their independent prognostic value for short-term mortality against the established MELD score.

Therefore, the primary aim of this study was to characterize the serum profiles of TNF-α, IL-6, IL-10, hs-CRP, and ESR across a spectrum of ALD severity defined by the MELD score. The secondary aim was to determine whether these inflammatory markers are independent predictors of 90-day all-cause mortality in patients with ALD. We hypothesized that a pro-inflammatory cytokine signature would correlate with disease severity and provide prognostic information beyond that offered by the MELD score alone.

Materials and Methods

Study Design and Population

This was a single-center, prospective cohort study conducted at a tertiary care centre.

A total of 200 participants were enrolled: 150 patients with ALD and 50 age- and sex-matched healthy controls. ALD patients were consecutively recruited from inpatient and outpatient clinics and stratified into three equal groups (n=50 each) based on their MELD score at enrollment: Low Severity (MELD < 15), Moderate Severity (MELD 15–25), and High Severity (MELD > 25).

Inclusion and Exclusion Criteria

Inclusion criteria for the ALD patient group were: (1) age between 18 and 70 years; (2) a clinical diagnosis of ALD confirmed by a history of significant alcohol consumption (defined as >60 g/day for men and >40 g/day for women for at least 5 years), compatible laboratory findings, and imaging evidence (ultrasound or CT scan), with liver biopsy results used where available; and (3) willingness to provide informed consent and participate in follow-up.

Exclusion criteria were: (1) evidence of other liver diseases, including viral hepatitis (HBsAg and anti-HCV negativity required), autoimmune hepatitis, hemochromatosis, or Wilson's disease; (2) presence of hepatocellular carcinoma; (3) an active, non-hepatic infection at the time of enrollment; (4) history of liver transplantation; (5) use of corticosteroids or other immunomodulatory drugs within 30 days of enrollment; and (6) severe extrahepatic comorbidities (e.g., end-stage renal disease on dialysis not related to hepatorenal syndrome, advanced heart failure). Healthy controls had no history of liver disease, significant alcohol consumption, or chronic inflammatory conditions.

Data Collection and Laboratory Procedures

At baseline, demographic data, clinical history, and physical examination findings were recorded for all participants. Standard laboratory tests, including complete blood count, liver function tests (bilirubin, AST, ALT, alkaline phosphatase, albumin), and renal function tests (creatinine), were performed. The MELD score was calculated using the standard formula.

For biomarker analysis, peripheral venous blood was collected in serum separator tubes. Samples were allowed to clot for 30 minutes at room temperature, then centrifuged at 1500 × g for 15 minutes. The resulting serum was aliquoted and stored at -80°C until analysis to prevent degradation.

Serum concentrations of TNF-α, IL-6, and IL-10 were measured using commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's

instructions. The lower limits of detection were 0.5 pg/mL for TNF-α, 0.7 pg/mL for IL-6, and 0.2 pg/mL for IL-10. High-sensitivity C-reactive protein (hs-CRP) was measured using a particle-enhanced immunoturbidimetric assay on an automated chemistry analyzer (Roche Diagnostics). Erythrocyte Sedimentation Rate (ESR) was determined using the Westergren method.

Outcome Assessment

The primary endpoint of the study was all-cause mortality at 90 days after enrollment. Patient outcomes were ascertained through review of electronic medical records and telephone contact with patients or their families if they did not have a follow-up hospital visit.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) based on their distribution, which was assessed using the Shapiro-Wilk test. Categorical variables were presented as frequencies and percentages.

Differences in baseline characteristics and biomarker levels among the four groups (Controls, Low-, Moderate-, and High-Severity ALD) were assessed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test for normally distributed data, or the Kruskal-Wallis H test with Dunn's post-hoc test for non-normally distributed data. The chi-square test was used for categorical variables.

To identify independent predictors of 90-day mortality, a multivariate Cox proportional hazards regression analysis was performed. Variables that were significant in univariate analysis ($p<0.10$) were included in the multivariate model. Due to their skewed distribution, cytokine and hs-CRP values were log-transformed before inclusion in the regression model. A two-tailed p-value < 0.05 was considered statistically significant for all analyses.

Results

Baseline Characteristics of the Study Population

A total of 150 ALD patients and 50 healthy controls were included in the final analysis. The baseline demographic and clinical characteristics of the participants are summarized in **Table 1**. The four groups were well-matched for age and sex. As expected, patients with ALD showed progressively worsening liver function with increasing MELD score, evidenced by significantly higher levels of AST, ALT, bilirubin, and INR, and lower levels of albumin compared to controls and to each other ($p<0.001$ for all).

Table 1. Baseline Demographic and Clinical Characteristics of the Study Population

Characteristic	Controls (n=50)	Low Severity ALD (MELD < 15) (n=50)	Moderate Severity ALD (MELD 15–25) (n=50)	High Severity ALD (MELD > 25) (n=50)	p-value
Age (years), mean ± SD	52.1 ± 9.8	53.5 ± 10.1	54.2 ± 8.9	55.0 ± 9.5	0.612
Sex (Male), n (%)	35 (70.0)	38 (76.0)	36 (72.0)	39 (78.0)	0.834
AST (U/L), mean ± SD	28.5 ± 6.1	115.4 ± 34.2	148.2 ± 45.1	185.6 ± 55.9	<0.001
ALT (U/L), mean ± SD	25.1 ± 7.2	68.3 ± 21.5	85.9 ± 30.7	102.4 ± 41.3	<0.001
Total Bilirubin (mg/dL), mean ± SD	0.7 ± 0.2	2.5 ± 1.1	8.9 ± 3.4	22.5 ± 7.8	<0.001
Albumin (g/dL),	4.2 ± 0.3	3.1 ± 0.5	2.6 ± 0.4	2.2 ± 0.3	<0.001

mean ± SD					
INR, mean ± SD	1.0 ± 0.1	1.4 ± 0.2	1.9 ± 0.4	2.8 ± 0.6	<0.001
MELD Score, mean ± SD	N/A	11.2 ± 2.4	19.8 ± 3.1	31.5 ± 5.2	<0.001

Serum Cytokine and Inflammatory Marker Levels

The serum levels of all measured inflammatory markers were significantly elevated in ALD patients compared to healthy controls and showed a stepwise increase corresponding to the severity of ALD (**Table 2**).

Mean IL-6 levels were highest in the High Severity ALD group (245.3 ± 58.7 pg/mL) and lowest in the control group (8.2 ± 2.1 pg/mL), with significant differences observed between all groups ($p<0.001$). A similar pattern was observed for TNF-α, with levels rising from 5.1 ± 1.8 pg/mL in controls to 85.2 ± 15.1 pg/mL in the high-severity group ($p<0.001$). The anti-inflammatory cytokine IL-10 was also elevated in ALD patients, but the magnitude of increase was less pronounced than for the pro-inflammatory cytokines. Conventional markers, hs-CRP and ESR, mirrored these trends, showing a strong positive correlation with disease severity.

Table 2. Serum Cytokine and Inflammatory Marker Levels by Study Group

Marker (unit)	Controls (n=50)	Low Severity ALD (MELD < 15) (n=50)	Moderate Severity ALD (MELD 15–25) (n=50)	High Severity ALD (MELD > 25) (n=50)	p-value
TNF-α (pg/mL)	5.1 ± 1.8	30.5 ± 8.2[a]	58.1 ± 12.5[ab]	85.2 ± 15.1[abc]	<0.001
IL-6 (pg/mL)	8.2 ± 2.1	45.6 ± 11.2[a]	112.8 ± 25.4[ab]	245.3 ± 58.7[abc]	<0.001
IL-10 (pg/mL)	3.5 ± 1.1	10.2 ± 3.5[a]	18.5 ± 5.1[ab]	25.8 ± 6.9[abc]	<0.001
hs-CRP (mg/L)	1.5 ± 0.8	12.1 ± 4.5[a]	28.9 ± 9.8[ab]	45.3 ± 12.6[abc]	<0.001
ESR (mm/hr)	10.2 ± 4.1	25.6 ± 8.2[a]	40.1 ± 10.5[ab]	58.7 ± 14.3[abc]	<0.001

Predictors of 90-Day Mortality

During the 90-day follow-up period, 28 of the 150 ALD patients (18.7%) died. Mortality was highest in the High Severity ALD group (n=19, 38.0%), followed by the Moderate Severity group (n=8, 16.0%) and the Low Severity group (n=1, 2.0%).

In the univariate Cox regression analysis, MELD score, Log IL-6, Log TNF-α, Log hs-CRP, Log IL-10, serum bilirubin, and INR were all significantly associated with 90-day mortality. In the multivariate Cox proportional hazards model, which included MELD score and log-transformed values of IL-6 and TNF-α, all three variables remained independent predictors of mortality (**Table 3**). A one-unit increase in MELD score was associated with a 14% increased risk of death (HR: 1.14). Importantly, after adjusting for the MELD score, each one-unit increase in Log IL-6 and Log TNF-α was associated with a 2.85-fold and 2.10-fold increased risk of 90-day mortality, respectively.

Table 3. Multivariate Cox Proportional Hazards Analysis for Predictors of 90-Day Mortality

Variable	Hazard Ratio (HR)	95% Confidence Interval (CI)	p-value
MELD Score (per 1-point increase)	1.14	1.07 – 1.22	<0.001
Log IL-6 (per 1-unit increase)	2.85	1.52 – 5.34	0.001
Log TNF-α (per 1-unit increase)	2.10	1.15 – 3.84	0.015

Discussion

This prospective study provides compelling evidence that systemic inflammation, as measured by serum cytokines, is closely linked to the severity of ALD and serves as a powerful independent predictor of short-term mortality. Our primary findings are twofold: first, the levels of pro-inflammatory cytokines TNF-α and IL-6, as well as hs-CRP, exhibit a robust, stepwise increase that correlates directly with ALD severity as defined by the MELD score. Second, and more critically, elevated baseline levels of IL-6 and TNF-α are independent predictors of 90-day mortality, even after adjusting for the MELD score.

The marked elevation of TNF-α and IL-6 in our high-severity ALD cohort is consistent with their known pathophysiological roles. Alcohol-induced gut dysbiosis and increased intestinal permeability lead to endotoxemia, which is a potent stimulus for Kupffer cell activation and subsequent cytokine production **[3]**, **[4]**. Our findings quantitatively demonstrate the magnitude of this inflammatory response, with IL-6 levels increasing over 30-fold in patients with MELD > 25 compared to healthy controls. This "cytokine storm" perpetuates liver injury by promoting hepatocyte apoptosis, recruiting other immune cells, and inducing a systemic inflammatory response syndrome, which is a major cause of death in patients with severe alcoholic hepatitis **[11]**. The results of our study align with previous reports, such as that by Vessal et al., who also found that IL-6 and IL-8 were strongly correlated with mortality in alcoholic hepatitis **[9]**. Our work extends these findings by including a broader spectrum of ALD severity and employing a rigorous multivariate model to establish their independent prognostic value.

The finding that IL-6 and TNF-α predict mortality independently of the MELD score is of significant clinical importance. The MELD score is an excellent measure of established hepato-renal dysfunction, but it is a relatively static reflection of organ failure **[7]**. In contrast, cytokine levels represent the dynamic, underlying biological process of inflammation that drives disease progression and precipitates acute-on-chronic liver failure. Our data suggest that integrating these biomarkers into clinical practice could provide a more complete and dynamic prognostic assessment. For instance, a patient with a moderate MELD score but exceptionally high IL-6 levels might be at a much higher short-term risk than predicted by MELD alone, potentially warranting more aggressive management or earlier consideration for liver transplantation. This supports the concept of moving towards a "MELD-Inflammation" score, as has been proposed by others **[8]**, **[12]**.
We also observed a significant, albeit more modest, rise in the anti-inflammatory cytokine IL-10 with increasing ALD severity. This likely reflects a compensatory anti-inflammatory response syndrome (CARS), an attempt by the immune system to counteract the overwhelming pro-inflammatory state **[6]**. However, in severe ALD, this compensatory mechanism is clearly inadequate to prevent organ damage and death, highlighting the profound immune dysregulation characteristic of the disease.

This study has several strengths, including its prospective design, the inclusion of a well-characterized and stratified patient cohort, and the simultaneous analysis of multiple key cytokines and conventional inflammatory markers. However, certain limitations must be acknowledged. First, this was a single-center study, which may limit the generalizability of our findings. Second, the 90-day follow-up period is appropriate for assessing short-term mortality but does not provide insight into long-term outcomes. Third, we did not serially measure cytokine levels, which could have provided further information on the dynamic nature of the inflammatory response to treatment or abstinence. Finally, while we controlled for major confounders, we did not assess genetic factors, such as polymorphisms in the TNF gene, which are known to influence cytokine production and ALD susceptibility **[13-16]**.

Future research should focus on validating these findings in larger, multi-center cohorts. Investigating the utility of these biomarkers for monitoring therapeutic response, particularly to anti-inflammatory agents, would be a logical next step. The development and validation of a new prognostic model incorporating IL-6 and/or TNF-α with the MELD score could represent a significant advance in the management of ALD.

Conclusion

In conclusion, this study demonstrates that serum levels of pro-inflammatory cytokines TNF-α and IL-6 are profoundly elevated in patients with alcoholic liver disease and strongly correlate with disease severity. Crucially, both markers serve as independent predictors of 90-day mortality, providing prognostic information that is complementary to the established MELD score. These findings underscore the central role of systemic inflammation in driving adverse outcomes in ALD and suggest that TNF-α and IL-6 are valuable biomarkers that could be integrated into clinical practice to enhance risk stratification and guide patient management.

References

1. Seitz HK, Bataller R, Cortez-Pinto H, et al. Alcoholic liver disease. Nat Rev Dis Primers. 2018;4(1):16.
2. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology. 2011;141(5):1572-1585.
3. Szabo G. Gut-liver axis in alcoholic liver disease. Gastroenterology. 2015;148(1):30-36.
4. Mandal P, Park PH, McMullen MR, Pratt BT, Nagy LE. The role of tumor necrosis factor-α in alcohol-induced liver injury. Alcohol. 2010;44(3):271-279.
5. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: from business as usual to disease. J Hepatol. 2016;64(6):1403-1415.
6. Szabo G, Saha B. Alcohol's effect on host defense. Alcohol Res. 2015;37(2):159-170.
7. Kamath PS, Wiesner RH, Malinchoc M, et al. A model to predict survival in patients with end-stage liver disease. Hepatology. 2001;33(2):464-470.
8. Cervoni JP, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, et al. C-reactive protein predicts short-term mortality in patients with cirrhosis. J Hepatol. 2012;56(6):1299-304.

9. Vessal G, Firoozi M, Firoozi M, et al. Proinflammatory Cytokines (IL-6, IL-8, and TNF-α) as Prognostic Markers of Mortality in Patients with Alcoholic Hepatitis. Alcohol Alcohol. 2021;56(2):189-194.
10. Bird GL, Sheron N, Goka AK, Alexander GJ, Williams RS. Increased plasma tumour necrosis factor in severe alcoholic hepatitis. Ann Intern Med. 1990;112(12):917-20.
11. Thursz MR, Richardson P, Allison M, et al. Prednisolone or pentoxifylline for alcoholic hepatitis. N Engl J Med. 2015;372(17):1619-1628.
12. Michelena J, Altamirano J, Abraldes JG, et al. Systemic inflammatory response and serum lipopolysaccharide levels predict multiple organ failure and death in alcoholic hepatitis. Hepatology. 2015;62(3):762-772.
13. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology. 1997;26(1):143-6.
14. Louvet A, Naveau S, Abdelnour M, et al. The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. Hepatology. 2007;45(6):1348-54.
15. Moreau R, Jalan R, Gines P, et al; CANONIC Study Investigators of the EASL-CLIF Consortium. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. Gastroenterology. 2013;144(7):1426-37, 1437.e1-9.
16. Crabb DW, Nagy LE, Szabo G, et al. Pathogenesis and treatment of alcoholic liver disease: 2019 update. Alcohol Clin Exp Res. 2020;44(4):787-805.