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*Original Article*

# Metabolomic Signatures Predictive of Fulminant Hepatic Failure In Autoimmune Hepatitis: A Cross-Sectional Study

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## ABSTRACT

Background: Autoimmune hepatitis may rarely present with fulminant hepatic failure, but routine liver tests may not fully characterize the metabolic disruption associated with this severe phenotype. Serum metabolomics may identify pathway-level disturbances related to energy metabolism, bile acid handling, amino acid imbalance, and mitochondrial stress. Objective: To compare serum metabolomic profiles between autoimmune hepatitis patients with and without fulminant hepatic failure and identify candidate metabolites associated with the fulminant phenotype. Methods: This cross-sectional study included 86 autoimmune hepatitis patients from tertiary care hospitals in Punjab, Pakistan. Thirty-two patients had fulminant hepatic failure and 54 did not. Clinical features, liver function tests, coagulation profile, immunoglobulin G, autoantibodies, and serum metabolomic profiles were assessed. Serum samples were analyzed using liquid chromatography–mass spectrometry. Group differences, fold changes, receiver operating characteristic curves, and adjusted associations were evaluated. Results: Patients with fulminant hepatic failure had higher bilirubin, ALT, AST, INR, and lower albumin. Lactate, pyruvate, phenylalanine, tyrosine, glutamine, total bile acids, and medium-chain acylcarnitines were higher in the fulminant group, while branched-chain amino acids were lower. Lactate showed the highest reported AUC, followed by total bile acids and phenylalanine. Conclusion: Fulminant hepatic failure in autoimmune hepatitis was associated with a distinct metabolomic pattern reflecting energy failure, bile acid overload, amino acid imbalance, and mitochondrial stress. These exploratory findings require validation in larger longitudinal studies. Keywords: Autoimmune hepatitis; Fulminant hepatic failure; Serum metabolomics; Lactate; Bile acids; Phenylalanine; Biomarkers; Pakistan.

## INTRODUCTION

Autoimmune hepatitis is a chronic immune-mediated inflammatory liver disease characterized by hepatocellular injury, elevated aminotransferases, increased serum immunoglobulin G, circulating autoantibodies, and compatible histological features when liver biopsy is feasible. Although many patients follow an indolent or relapsing course, autoimmune hepatitis may also present acutely with severe jaundice, coagulopathy, and hepatic encephalopathy, creating substantial diagnostic and therapeutic urgency. Current international guidelines emphasize early recognition, exclusion of competing liver diseases, and timely immunosuppressive management because delayed diagnosis may increase the risk of severe hepatic dysfunction and poor clinical outcomes (1-4).

The epidemiology of autoimmune hepatitis varies across regions, and global estimates remain less complete for low- and middle-income countries. A recent systematic review has highlighted wide variation in reported incidence and prevalence, partly reflecting differences in case ascertainment, diagnostic access, and health-system capacity (5). In Pakistan, available studies have documented autoimmune liver disease in both adult and pediatric populations, including variation in clinical presentation, autoantibody profiles, and genetic associations, but local evidence remains limited compared with data from high-resource settings (6-8). This gap is clinically important because patients in tertiary care hospitals may present late after prolonged jaundice, nonspecific constitutional symptoms, or initial evaluation for viral hepatitis, drug-induced liver injury, or other common hepatobiliary disorders. In such settings, improved tools for identifying severe biological injury in autoimmune hepatitis may support earlier escalation of care.

Fulminant hepatic failure, commonly described within the broader clinical framework of acute liver failure, represents rapid deterioration of liver function with jaundice, coagulopathy, and hepatic encephalopathy in a patient without known advanced cirrhosis. In autoimmune hepatitis, fulminant presentation is less frequent than chronic presentation but carries high clinical significance because deterioration may occur over a short period and decisions regarding corticosteroid treatment, intensive monitoring, supportive management, and transplant referral may be time-sensitive. Existing diagnostic approaches rely on symptoms, liver biochemistry, coagulation profile, albumin, immunoglobulin G, autoantibodies, viral markers, clinical scoring systems, and liver histology where possible; however, these parameters may not fully capture the underlying metabolic disturbance associated with acute hepatic collapse (9-18).

The liver has a central role in energy metabolism, amino acid homeostasis, bile acid synthesis and clearance, lipid handling, mitochondrial oxidation, and detoxification. Severe hepatic injury may therefore produce detectable changes in circulating small molecules before or alongside overt clinical deterioration. Metabolomics provides a systems-level assessment of these small molecules, including amino acids, bile acids, organic acids, acylcarnitines, phospholipids, sphingolipids, lactate, pyruvate, and other energy-related metabolites (19). Previous metabolomic studies in advanced liver disease and acute-on-chronic liver failure have shown that disturbances in energy metabolism, aromatic amino acids, bile acids, acylcarnitines, and related pathways may distinguish patients with more severe hepatic dysfunction from those with less advanced disease (20-22).

Despite these advances, serum metabolomic evidence specific to autoimmune hepatitis complicated by fulminant hepatic failure remains limited. Most autoimmune hepatitis guidance focuses on diagnosis, induction and maintenance immunosuppression, remission, relapse, and long-term monitoring, whereas fewer studies have examined whether metabolic signatures can characterize the severe fulminant phenotype at presentation. This is a relevant knowledge gap for Punjab, Pakistan, where delayed presentation and limited availability of advanced liver support or transplant pathways may increase the need for clinically interpretable markers that complement routine liver tests. A metabolomic approach may help clarify whether autoimmune hepatitis with fulminant hepatic failure is associated with a distinct pattern of energy failure, bile acid accumulation, amino acid imbalance, and mitochondrial stress rather than merely higher aminotransferase levels.

Therefore, this cross-sectional study was conducted to compare serum metabolomic profiles between patients with autoimmune hepatitis with fulminant hepatic failure and those without fulminant hepatic failure. The primary objective was to identify serum metabolites associated with the fulminant hepatic failure phenotype in autoimmune hepatitis. The secondary objective was to explore the discriminatory performance of selected candidate metabolites, including lactate, total bile acids, phenylalanine, and related metabolic markers, for differentiating autoimmune hepatitis patients with fulminant hepatic failure from those without fulminant hepatic failure.

## MATERIALS AND METHODS

This cross-sectional hospital-based study was conducted in selected tertiary care hospitals of Punjab, Pakistan, to compare serum metabolomic patterns among patients diagnosed with autoimmune hepatitis with and without fulminant hepatic failure. Data and blood samples were collected at a single hospital visit or admission, preferably before major treatment modification when clinically feasible. The cross-sectional design was selected to evaluate metabolic differences associated with the fulminant hepatic failure phenotype at presentation rather than to establish temporal prediction or causal progression.

Patients aged 12 years and above with diagnosed autoimmune hepatitis were eligible for inclusion. The diagnosis of autoimmune hepatitis was based on a composite clinical and laboratory assessment including compatible clinical features, elevated aminotransferases, raised serum immunoglobulin G, positive autoimmune serology, exclusion of viral hepatitis, and simplified autoimmune hepatitis criteria where available. Liver biopsy findings were recorded when biopsy had been performed as part of clinical care, but biopsy was not required in clinically unstable patients. Participants were classified into two groups. Group A included autoimmune hepatitis patients with fulminant hepatic failure, defined as acute worsening of liver function with jaundice, coagulopathy with raised INR, and altered mental status or hepatic encephalopathy in the absence of known established cirrhosis. Group B included autoimmune hepatitis patients without fulminant hepatic failure, defined as autoimmune hepatitis without encephalopathy and without marked acute liver failure features at the time of assessment.

Patients were excluded if they had active hepatitis B infection, active hepatitis C infection, Wilson disease, alcoholic liver disease, drug-induced liver injury, hepatocellular carcinoma, pregnancy, chronic kidney disease stage 4 or 5, severe active bacterial sepsis at the time of sampling, or previous liver transplantation. These exclusions were applied to reduce diagnostic misclassification and minimize major non-autoimmune causes of hepatic failure or metabolomic distortion. Non-probability consecutive sampling was used, and all eligible patients presenting during the study period were invited to participate. This approach was used because fulminant hepatic failure in autoimmune hepatitis is relatively uncommon and random sampling would be impractical in a hospital-based severe liver disease population.

After informed consent, demographic and clinical data were collected on a structured proforma. Recorded variables included age, gender, residence, symptom duration, jaundice, abdominal pain, fever, fatigue, vomiting, confusion or altered sensorium, bleeding tendency, previous autoimmune disease, previous diagnosis of autoimmune hepatitis, and prior use of corticosteroids or other immunosuppressive drugs. Routine laboratory variables were extracted from hospital records and included complete blood count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, direct bilirubin, serum albumin, prothrombin time, INR, serum creatinine, serum sodium, serum immunoglobulin G, antinuclear antibody, anti-smooth muscle antibody, anti-liver kidney microsomal antibody, and viral hepatitis markers. Where available, liver biopsy findings were recorded but were not used as a compulsory eligibility requirement in patients with acute clinical instability.

Five milliliters of venous blood was collected from each participant using standard aseptic technique. Fasting sampling was preferred where clinically possible; however, non-fasting samples were accepted in emergency fulminant hepatic failure cases when delaying blood collection was not clinically appropriate. Blood was collected in plain tubes, allowed to clot at room temperature, and centrifuged at 3000 rpm for 10 minutes. The separated serum was aliquoted into coded tubes to avoid repeated freeze–thaw cycles and stored at −80°C until metabolomic analysis. Patient identifiers were not used on laboratory aliquots, and each specimen was handled using a study code to preserve confidentiality and reduce analytical bias.

Serum metabolomic profiling was performed using liquid chromatography–mass spectrometry. Before analysis, serum proteins were precipitated using cold methanol. Samples were vortexed, kept on ice, centrifuged, and the clear supernatant was transferred to autosampler vials for analysis. Quality control samples were prepared by pooling small aliquots from study specimens and were injected at regular intervals during the analytical run to assess instrument stability. Blank samples were also included to identify possible contamination. The candidate metabolites of interest included lactate, pyruvate, phenylalanine, tyrosine, glutamine, total bile acids, medium-chain acylcarnitines, branched-chain amino acids, phospholipids, sphingolipids, organic acids, and other energy-related metabolites, selected because of their biological relevance to hepatic energy metabolism, amino acid balance, bile acid handling, lipid oxidation, and mitochondrial stress.

The primary exposure classification was the presence or absence of fulminant hepatic failure among patients with autoimmune hepatitis. The primary metabolomic variables were serum levels of candidate metabolites involved in energy metabolism, bile acid metabolism, amino acid metabolism, and fatty acid oxidation. Routine biochemical severity markers included bilirubin, aminotransferases, INR, albumin, and immunoglobulin G. Hepatic encephalopathy was treated as a defining clinical feature of fulminant hepatic failure rather than as an independent comparative outcome. Potential confounders considered in adjusted analysis included age, gender, bilirubin, INR, and albumin, because these variables may influence the relationship between metabolite levels and fulminant hepatic failure classification.

Data were entered and analyzed using statistical software. Continuous variables were summarized as mean and standard deviation for approximately normally distributed data and as median with interquartile range for skewed data where appropriate. Categorical variables were summarized as frequency and percentage. Group comparisons between autoimmune hepatitis patients with and without fulminant hepatic failure were performed using the independent-samples t-test or Mann–Whitney U test for continuous variables according to distribution, and the chi-square test or Fisher exact test for categorical variables as appropriate. Metabolites showing group differences were further assessed using fold change and receiver operating characteristic curve analysis to explore their discriminatory performance for identifying the fulminant hepatic failure phenotype within this cross-sectional dataset.

Area under the receiver operating characteristic curve was calculated for selected candidate metabolites. Multivariable logistic regression was used to assess whether key metabolites remained associated with fulminant hepatic failure after adjustment for age, gender, bilirubin, INR, and albumin. Results were interpreted as exploratory associations and discriminatory estimates rather than longitudinal prediction. A p-value less than 0.05 was considered statistically significant. All analyses were planned to maintain consistency between the study design, variable definitions, and reporting of cross-sectional associations.

## RESULTS

A total of 86 patients diagnosed with autoimmune hepatitis were included in the analysis. Of these, 32 patients were classified as having autoimmune hepatitis with fulminant hepatic failure and 54 patients were classified as having autoimmune hepatitis without fulminant hepatic failure. The overall mean age was 34.7 ± 12.6 years. Female predominance was observed in the total cohort and in both clinical groups.

Patients with autoimmune hepatitis and fulminant hepatic failure had more severe biochemical derangement than those without fulminant hepatic failure. Total bilirubin was higher in the FHF group than in the non-FHF group, with mean values of 18.6 ± 6.9 mg/dL and 7.8 ± 3.4 mg/dL, respectively. Markers of hepatocellular injury were also higher in the FHF group, including ALT at 624 ± 241 U/L compared with 386 ± 196 U/L and AST at 711 ± 268 U/L compared with 421 ± 214 U/L. Synthetic dysfunction was more pronounced in the FHF group, with a higher INR of 3.1 ± 0.8 and lower serum albumin of 2.6 ± 0.5 g/dL. Serum IgG was elevated in both groups but was higher among patients with FHF.

*Table 1. Baseline Clinical and Biochemical Characteristics of Patients With Autoimmune Hepatitis According to Fulminant Hepatic Failure Status*

| Variable | AIH With FHF (n = 32) | AIH Without FHF (n = 54) | p-value |
|---|---|---|---|
| Age, years | 32.9 ± 11.8 | 35.8 ± 13.1 | 0.301 |
| Female gender, n (%) | 24 (75.0) | 39 (72.2) | 0.779 |
| Total bilirubin, mg/dL | 18.6 ± 6.9 | 7.8 ± 3.4 | <0.001 |
| ALT, U/L | 624 ± 241 | 386 ± 196 | <0.001 |
| AST, U/L | 711 ± 268 | 421 ± 214 | <0.001 |
| INR | 3.1 ± 0.8 | 1.4 ± 0.3 | <0.001 |
| Serum albumin, g/dL | 2.6 ± 0.5 | 3.4 ± 0.6 | <0.001 |
| Serum IgG, mg/dL | 2710 ± 684 | 2385 ± 590 | 0.021 |

*Table 2. Serum Metabolomic Profile of Autoimmune Hepatitis Patients According to Fulminant Hepatic Failure Status*

| Metabolite | AIH With FHF (n = 32) | AIH Without FHF (n = 54) | Fold Change | p-value |
|---|---|---|---|---|
| Lactate, mmol/L | 4.8 ± 1.6 | 2.1 ± 0.8 | 2.28 | <0.001 |
| Pyruvate, µmol/L | 156 ± 48 | 92 ± 31 | 1.69 | <0.001 |
| Phenylalanine, µmol/L | 118 ± 34 | 68 ± 21 | 1.73 | <0.001 |
| Tyrosine, µmol/L | 104 ± 29 | 61 ± 18 | 1.70 | <0.001 |
| Glutamine, µmol/L | 842 ± 210 | 612 ± 165 | 1.38 | <0.001 |
| Total bile acids, µmol/L | 72 ± 26 | 28 ± 14 | 2.57 | <0.001 |
| Medium-chain acylcarnitines, µmol/L | 3.2 ± 1.1 | 1.4 ± 0.6 | 2.28 | <0.001 |
| Branched-chain amino acids, µmol/L | 286 ± 74 | 392 ± 89 | 0.72 | <0.001 |

Values are presented as mean ± SD unless otherwise indicated. AIH, autoimmune hepatitis; FHF, fulminant hepatic failure.

Distinct metabolomic differences were observed between the two groups. Lactate was more than two-fold higher in the FHF group than in the non-FHF group, with mean values of 4.8 ± 1.6 mmol/L and 2.1 ± 0.8 mmol/L, respectively. Total bile acids showed the largest fold difference, with values of 72 ± 26 µmol/L in the FHF group and 28 ± 14 µmol/L in the non-FHF group. Aromatic amino acids were also higher in the FHF group, including phenylalanine at 118 ± 34 µmol/L compared with 68 ± 21 µmol/L and tyrosine at 104 ± 29 µmol/L compared with 61 ± 18 µmol/L. Medium-chain acylcarnitines were higher in the FHF group, while branched-chain amino acids were lower, with mean values of 286 ± 74 µmol/L in the FHF group and 392 ± 89 µmol/L in the non-FHF group.

*Table 3. Discriminatory Performance of Selected Serum Metabolites for Fulminant Hepatic Failure in Autoimmune Hepatitis*

| Metabolite | AUC |
|---|---|
| Lactate | 0.86 |
| Total bile acids | 0.84 |
| Phenylalanine | 0.81 |
| Medium-chain acylcarnitines | 0.78 |

AUC, area under the receiver operating characteristic curve.

Receiver operating characteristic analysis showed that lactate had the highest discriminatory performance for differentiating autoimmune hepatitis patients with fulminant hepatic failure from those without fulminant hepatic failure, with an AUC of 0.86. Total bile acids also showed strong discrimination with an AUC of 0.84, followed by phenylalanine with an AUC of 0.81. Medium-chain acylcarnitines showed moderate-to-good discriminatory ability with an AUC of 0.78. These findings indicate that markers reflecting energy metabolism, bile acid accumulation, amino acid imbalance, and fatty acid oxidation stress were associated with the FHF phenotype in this cross-sectional dataset.

*Table 4. Summary of Candidate Metabolites Associated With Fulminant Hepatic Failure After Adjustment*

| Metabolite | Adjustment Variables | Direction of Association |
|---|---|---|
| Lactate | Age, gender, bilirubin, INR, albumin | Positive |
| Total bile acids | Age, gender, bilirubin, INR, albumin | Positive |
| Phenylalanine | Age, gender, bilirubin, INR, albumin | Positive |

In multivariable logistic regression analysis, lactate, total bile acids, and phenylalanine remained positively associated with fulminant hepatic failure after adjustment for age, gender, bilirubin, INR, and albumin. Lactate showed the strongest reported association, followed by total bile acids and phenylalanine. Because adjusted effect estimates and confidence intervals were not available, these findings should be interpreted as exploratory adjusted associations rather than validated predictive estimates.

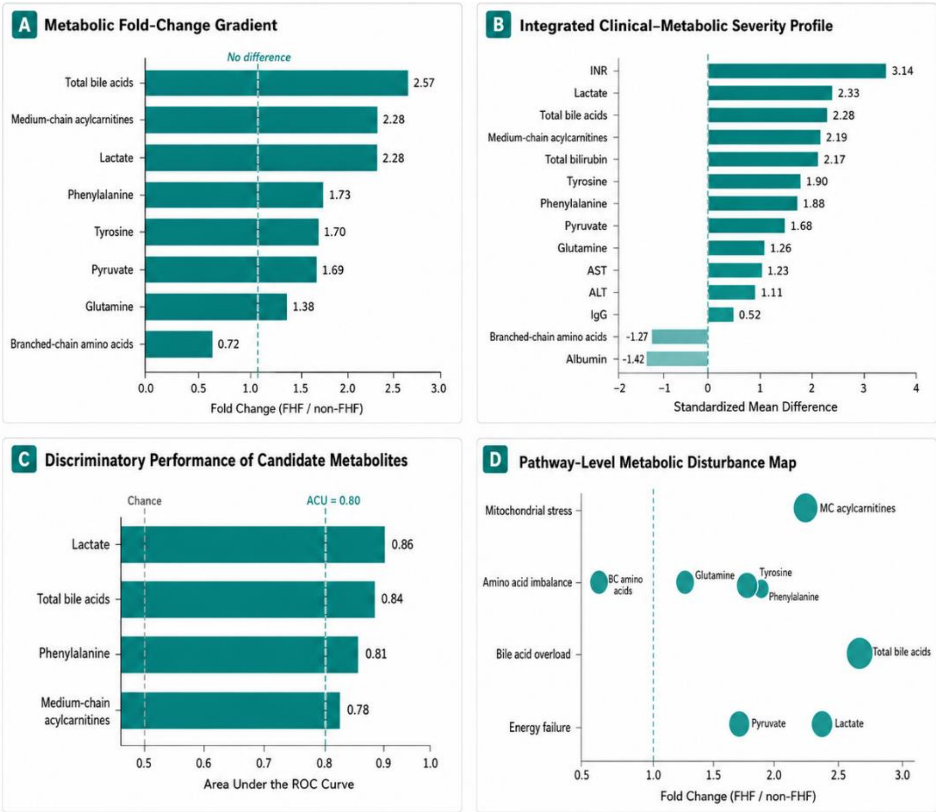


*Figure 1 Metabolomic disturbance associated with fulminant hepatic failure in autoimmune hepatitis. Panel A shows metabolite fold-change gradients between autoimmune hepatitis patients with fulminant hepatic failure and those without fulminant hepatic failure. Panel B integrates standardized clinical and metabolic group differences. Panel C ranks the reported discriminatory performance of selected metabolites using AUC values. Panel D maps fold-change magnitude across biologically relevant pathway domains. The strongest disturbance was observed for total bile acids, lactate, medium-chain acylcarnitines, and aromatic amino acids, while branched-chain amino acids were lower in the fulminant hepatic failure group. All visualized values were derived from reported aggregate means, standard deviations, fold changes, and AUCs; no individual-level or simulated data were used.*

Overall, patients with autoimmune hepatitis and fulminant hepatic failure demonstrated a consistent pattern of severe biochemical dysfunction and metabolomic disturbance. The FHF group showed higher bilirubin, aminotransferases, INR, lactate, pyruvate, aromatic amino acids, glutamine, total bile acids, and medium-chain acylcarnitines, along with lower albumin and branched-chain amino acids. This profile suggests that the fulminant phenotype of autoimmune hepatitis is associated with combined hepatocellular injury, impaired synthetic function, energy metabolism disruption, bile acid accumulation, amino acid imbalance, and possible mitochondrial stress.

## DISCUSSION

This cross-sectional study demonstrated that autoimmune hepatitis patients with fulminant hepatic failure had a substantially different clinical, biochemical, and metabolomic profile compared with autoimmune hepatitis patients without fulminant hepatic failure. The FHF group showed higher bilirubin, aminotransferases, INR, and lower albumin, indicating more severe hepatocellular injury and impaired hepatic synthetic function. These findings are consistent with the clinical framework of acute liver failure, in which rapid deterioration of liver function is characterized by jaundice, coagulopathy, and hepatic encephalopathy in the absence of known advanced cirrhosis (23,24). Although these conventional markers are essential for clinical recognition, the present findings suggest that the fulminant phenotype of autoimmune hepatitis is also accompanied by broader metabolic disruption involving energy metabolism, bile acid handling, amino acid balance, and mitochondrial stress.

The female predominance observed in both groups aligns with the established epidemiological pattern of autoimmune hepatitis, which is more commonly reported in females and may present across a wide age range with variable severity (1,12,15). Local studies from Pakistan have also shown that autoimmune liver diseases are present in tertiary care populations and may be recognized after substantial clinical progression (6-8). This context is important because autoimmune hepatitis may be under-recognized in settings where viral hepatitis, drug-induced liver injury, and other hepatobiliary conditions are common differential diagnoses. The present study adds local evidence from Punjab by showing that autoimmune hepatitis with fulminant hepatic failure is not only biochemically severe but also metabolically distinct from non-fulminant autoimmune hepatitis.

Among the studied metabolites, lactate showed one of the strongest differences between the groups and the highest reported discriminatory performance, with an AUC of 0.86. Lactate was more than two-fold higher in the FHF group, suggesting impaired hepatic lactate clearance and altered energy metabolism. The liver plays a central role in lactate utilization and gluconeogenesis; therefore, severe hepatocellular dysfunction may lead to lactate accumulation through reduced clearance, increased anaerobic metabolism, and mitochondrial impairment. Similar disturbances in energy metabolism have been described in metabolomic studies of advanced liver disease and acute-on-chronic liver failure, where metabolic signatures reflect the severity of hepatic dysfunction beyond routine biochemical tests (20-22). In the present study, lactate remained positively associated with fulminant hepatic failure after adjustment for age, gender, bilirubin, INR, and albumin, supporting its potential role as an exploratory marker of metabolic severity in autoimmune hepatitis.

The rise in pyruvate alongside lactate further supports disruption of energy-related pathways. Lactate and pyruvate are closely linked through glycolysis and mitochondrial oxidative metabolism, and their concurrent elevation may indicate reduced mitochondrial processing of energy substrates during severe hepatic injury. Although autoimmune hepatitis is primarily immune-mediated, progression to fulminant hepatic failure appears to involve downstream metabolic failure that extends beyond inflammatory hepatocellular injury alone. This finding is biologically plausible because the liver is a major site of oxidative metabolism, substrate cycling, and systemic energy regulation. However, because the study design was cross-sectional, these findings should be interpreted as metabolic associations observed at presentation rather than evidence that lactate or pyruvate temporally predicts subsequent fulminant hepatic failure.

Total bile acids showed the largest fold difference between groups and demonstrated strong discriminatory performance, with an AUC of 0.84. Bile acids are synthesized, conjugated, transported, and cleared by the liver, and their accumulation reflects impaired hepatocellular transport, cholestatic stress, and reduced hepatic clearance. Although autoimmune hepatitis is classically hepatocellular rather than primarily cholestatic, severe inflammatory injury may impair bile acid handling and contribute to systemic metabolic toxicity. The marked elevation of total bile acids in the FHF group suggests that bile acid overload may be a relevant component of the fulminant phenotype. This finding supports the value of metabolomic profiling because pathway-level changes may reveal aspects of liver failure biology that are not fully captured by aminotransferases alone (19,21,22).

Aromatic amino acids, particularly phenylalanine and tyrosine, were also higher in patients with fulminant hepatic failure, while branched-chain amino acids were lower. This pattern is clinically meaningful because liver dysfunction alters amino acid metabolism, and imbalance between aromatic amino acids and branched-chain amino acids has been linked to severe hepatic dysfunction and encephalopathy-related metabolic disturbance. In this study, phenylalanine showed an AUC of 0.81 and remained positively associated with fulminant hepatic failure after adjustment for key clinical variables.

The reduction in branched-chain amino acids in the FHF group further supports an amino acid imbalance consistent with advanced hepatic metabolic stress. These findings align with metabolomic literature indicating that amino acid disturbances may reflect altered nitrogen handling, impaired hepatic metabolism, and neuro-metabolic consequences of severe liver failure (19,22).

Medium-chain acylcarnitines were higher in the FHF group and showed an AUC of 0.78. Acylcarnitine accumulation may reflect incomplete fatty acid oxidation and mitochondrial stress, both of which are relevant to severe liver injury. The liver is central to lipid oxidation and mitochondrial energy production, and disruption of these pathways may produce accumulation of acylcarnitine intermediates. This finding suggests that autoimmune hepatitis-related fulminant hepatic failure may involve mitochondrial and lipid oxidation stress in addition to immune-mediated hepatocellular damage. The combined pattern of higher lactate, pyruvate, bile acids, aromatic amino acids, glutamine, and medium-chain acylcarnitines, with lower branched-chain amino acids, indicates a coordinated metabolic disturbance rather than isolated metabolite variation.

The integrated clinical and metabolomic profile supports the interpretation that fulminant hepatic failure in autoimmune hepatitis represents a multi-pathway deterioration involving biochemical severity and systemic metabolic collapse. Routine markers such as bilirubin, INR, albumin, ALT, and AST remain essential for clinical diagnosis and severity assessment, but the metabolomic findings may help characterize the biological depth of liver failure. Importantly, these findings should be interpreted as exploratory and hypothesis-generating. The reported AUC values indicate within-sample discriminatory ability, not validated prediction. For these metabolites to be used clinically, future studies would need prespecified thresholds, sensitivity, specificity, positive and negative predictive values, calibration, internal validation, and external validation in independent cohorts.

This study has several limitations. First, the cross-sectional design prevents assessment of temporal prediction, causality, treatment response, survival, transplant-free survival, or progression from non-fulminant autoimmune hepatitis to fulminant hepatic failure. Second, the study was hospital-based and limited to tertiary care settings in Punjab, so findings may not generalize to community-based, mild, or early autoimmune hepatitis populations. Third, some emergency samples were accepted in non-fasting conditions, which may influence metabolite levels. Fourth, the fulminant hepatic failure group included only 32 patients, limiting statistical power for multivariable modeling and increasing the risk of model instability. Fifth, metabolomic reporting requires further technical detail, including analyte definitions, platform specifications, normalization procedures, batch correction, missing data handling, and multiple-comparison correction. Sixth, adjusted regression results were described without reported odds ratios, confidence intervals, p-values, or model diagnostics, which limits interpretability. Finally, the composite metabolite categories, including medium-chain acylcarnitines and branched-chain amino acids, require clearer analytical definition before reproducibility can be ensured.

Despite these limitations, the study provides useful local evidence and identifies biologically plausible candidate metabolites associated with fulminant hepatic failure in autoimmune hepatitis. Lactate, total bile acids, phenylalanine, and medium-chain acylcarnitines appear to reflect clinically relevant pathways of energy failure, bile acid accumulation, amino acid imbalance, and mitochondrial stress. Future multicenter studies should include longitudinal sampling, standardized fasting protocols where feasible, detailed LC-MS reporting, external validation cohorts, and outcome-linked analyses including encephalopathy grade, steroid response, intensive care requirement, transplant referral, mortality, and transplant-free survival. Such work would clarify whether these metabolites can move from exploratory association toward clinically useful risk stratification.

## CONCLUSION

Autoimmune hepatitis patients with fulminant hepatic failure demonstrated a distinct serum metabolomic profile compared with autoimmune hepatitis patients without fulminant hepatic failure.

The FHF group had more severe biochemical dysfunction, including higher bilirubin, aminotransferases, INR, and lower albumin, along with marked elevations in lactate, pyruvate, phenylalanine, tyrosine, glutamine, total bile acids, and medium-chain acylcarnitines, while branched-chain amino acids were lower. Lactate, total bile acids, and phenylalanine showed the strongest reported discriminatory performance and remained positively associated with fulminant hepatic failure after adjustment for key clinical variables. These findings suggest that AIH-related fulminant hepatic failure is associated with multi-pathway metabolic disruption involving energy failure, bile acid overload, amino acid imbalance, and mitochondrial stress. However, because the study was cross-sectional, these metabolites should be considered exploratory candidate markers requiring validation in larger longitudinal and multicenter studies before clinical implementation.

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