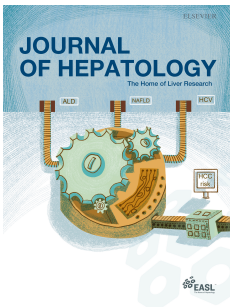

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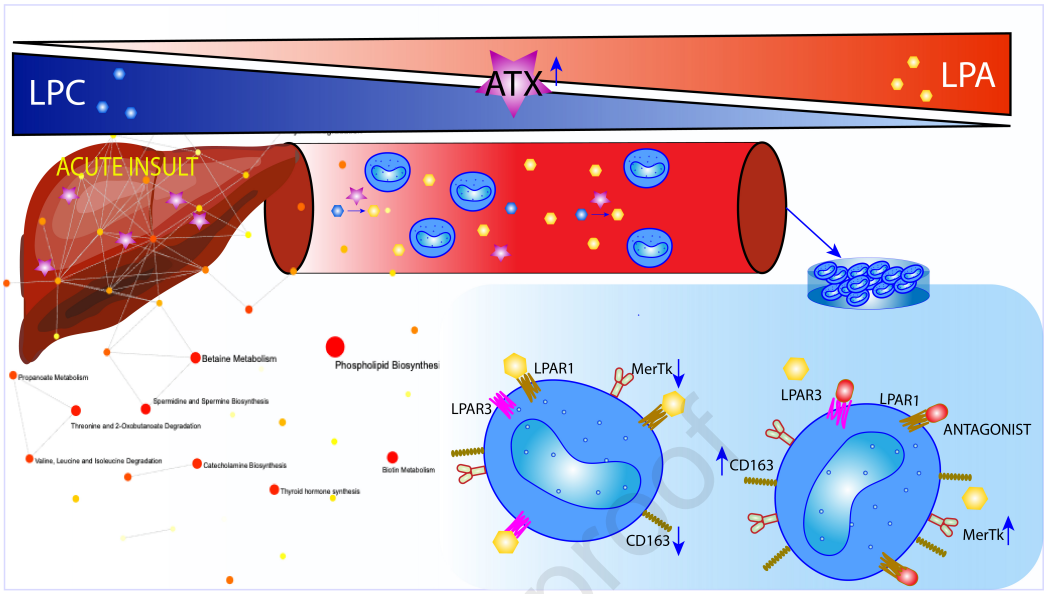

Lysophosphatidylcholines modulate immunoregulatory checkpoints in peripheral monocytes and are associated with mortality in people with acute liver failure

Short title: Active lipids modulate monocytes in ALF

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Conflict of interest

Muireann Coen is an employee of AstraZeneca and has stock ownership and/or stock options or interests in the company. William Bernal is consultant for Versantis AG and Pioneering Medicine VII, Inc.

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Authors Contribution

FMT, MJWM, study concept and design; FMT, RZ, FA, SM, EJ, AC, MC, SN, AS, CB acquisition of data; FMT,MJWM, RZ, MC, IW, EH, PM, MC, RM, ET, SRA analysis and interpretation of data; FMT, MJWM, drafting of the manuscript; FMT, MJWM, MC, IW, JW, WB, KM, VCP, ET critical revision of the manuscript for important intellectual content; FMT, MJWM, RZ, SA statistical analysis; MJWM, VCP, KM, ET obtained funding; FMT, SN, SM, administrative, technical, or material support; MJWM, study supervision.

Data availability statement

Data can be made available at reasonable request and uploaded on acceptance

Keywords: lysophosphatidylcholine, acute liver failure, immune checkpoint, LPAR, MerTK, CD163, RNA sequencing, Monocytes

Abstract

Background and Aims: Acute liver failure (ALF) is a life-threatening disease characterised by high-grade inflammation and immunoparesis with a high incidence of death from sepsis. Here, we aimed to describe the metabolic dysregulation in ALF and determine whether systemic immune responses are modulated *via* the lysophosphatidylcholine(LPC)-autotaxin(ATX)-lysophosphatidylcholinic acid (LPA) pathway.

Methods: 96 ALF patients, 71 healthy controls (HC), 104 patients with cirrhosis and 31 septic patients were recruited. The pathways of interest were identified based on multivariate statistical analysis of proton nuclear magnetic resonance (^{1}HNMR) spectroscopy, untargeted ultraperformance liquid chromatography-mass spectrometry (UPLC-MS)-based lipidomics and validated with a targeted metabolomics panel. Peripheral blood mononuclear cells were cultured with LPA 16:0, 18:0, 18:1, and their immune checkpoint surface expression was assessed by flow cytometry. LPA receptor (LPAR) transcript-level expression of monocytes was investigated and the effect of LPAR antagonism was also examined *in vitro*.

Results: LPC 16:0 was found highly discriminant between ALF and HC. There was an increase in ATX and LPA in ALF compared to HC and sepsis. LPCs 16:0, 18:0 and 18:1 were reduced in ALF patients with poor prognosis. Treatment of monocytes with LPA 16:0 increased their PD-L1 expression and reduced CD155, CD163, MerTK levels, without effect on T and NK/CD56+T cells immune checkpoints. LPAR1 and 3 antagonism in culture reversed the LPA effect on monocyte expression of MerTK and CD163. MerTK and CD163, but not LPARs genes, were differently expressed and upregulated in monocytes from ALF patients compared to controls.

Conclusion: Reduced amounts of LPCs are biomarkers of poor prognosis in patients with ALF. The LPC-ATX-LPA axis appears to modulate innate immune response in ALF *via* LPAR1 and LPAR3. Further investigations are required to identify novel therapeutic agents targeting these receptors.

Impact and implications

Liver disease is the 5th leading cause of death in the UK and rising in incidence. Acute liver failure occurs on the background of normal liver function and mostly in young adults. Acute admissions to hospital and intensive care units are rising in the UK and worldwide.

We identified a metabolic signature of acute liver failure and investigated the immunometabolic role of the Lysophosphatdylcholine(LPC)-Autotaxin (ATX)-Lysophosphatidylcholinic acid (LPA) pathway in order to find a mechanistic explanation for monocyte behaviour and find possible therapeutic target(s) to modulate the systemic immune response in ALF. At present, no selective immune based therapies exist. We were able to modulate monocyte phenotype and function in vitro and aim to extend findings to murine models of ALF before could apply this treatment to patients. Future therapies may be based on the enhancement of resolution through metabolic modulation and therefore the role of specific lipids in this pathway require elucidation and the relative merits of ATX inhibition, LPAR blockade or lipid-based therapies answered. This application aims to make a step change in meeting this knowledge gap and definitively elucidate these immune-metabolic pathways using an experimental medicine approach, thus finding the most effective therapeutic targets.

Introduction

Acute liver failure (ALF) is a rare life-threatening disease characterised by acute derangement of liver synthetic function with coagulopathy and altered levels of consciousness from hepatic encephalopathy (HE) in people without previous liver disease.[1] ALF is characterised by intense systemic inflammation and parallel impairment of innate and adaptive antimicrobial immune responses (immunoparesis) with a high incidence of death from sepsis and multiorgan failure.[2] In this context, immune cell function is often suppressed by multiple mechanisms, including inhibitory signalling *via* immune checkpoint pathways, such as Programmed cell death 1 (PD-1)[3] and Cytotoxic T-lymphocyte-associated protein 4 (CTLA4).[4] However, no selective immune based therapies exist for ALF.

The association of ALF and sepsis is due to a switch of the innate immune system to a regulatory, anti-inflammatory mode during the first week of illness as systemic inflammation subsides. Expression of MerTK (mediating the efferocytosis of apoptotic cell) and CD163 (a scavenger receptor) increases in peripheral monocytes and hepatic macrophages favour tissue repair at the expense of a higher risk of sepsis[5,6]. We recently demonstrated that PD-1 blockade restored human monocyte functionality *in vitro* while PD-1-deficient mice and anti-PD-1-treated mice with liver injury showed improved Kupffer cell bacterial clearance and protection from sepsis.[3] Immune checkpoint inhibition has however, several safety concerns in these patients given the potential for worsening liver injury.[7] The mechanisms leading to monocyte reprogramming and immune checkpoint overexpression are not yet defined and modulating them to prevent sepsis and to safely promote hepatic regeneration is an unmet clinical need in ALF. Importantly, it was recently proposed that metabolic factors may evoke monocyte reprogramming in acute-on-chronic liver failure (ACLF)[8], but whether this finding extends to ALF remains unknown.

The liver, as a major metabolic organ, is responsible for the metabolism of many exogenous compounds and synthesis of endogenous metabolites. These factors make untargeted metabolomics/lipidomics, as well as more targeted methods for e.g., acylcarnitines, and bile acids[9] as markers of mitochondrial dysfunction and hepatotoxicity, respectively, a useful approach for its investigation of function. This was recently highlighted in studies of paracetamol-related ALF by providing a mean of gaining a metabolic signature of disease and for highlighting pathways for mechanistic and therapeutic exploration[9].

More recently the prognostic role of lysophosphatidylcholines (LPCs) in ACLF, and their potential in monocyte functional modulation has been shown.[8] Lysophosphatidylcholinic acid (LPA) and LPC are the most studied lysoglycerophospholipids species modulating acute and chronic inflammatory processes. LPA originate from the hydrolysation of membrane phospholipids, including phosphatidylcholine (PC), phosphatifylethanolamine (PE), and phosphatidylserine (PS), to lysophospholipids performed by phospholipases A1 (PLA1) and 2 (PLA2). Then a lysophospholipase D, named autotaxin (ATX), converts LPC to LPA.

The LPC-ATX-LPA axis has been reported to exert a its effect through LPA_{1-3} receptors and LPA_{4-6} receptors that belong to the endothelial gene (EDG) and non-endothelial gene families respectively.[10]

These receptors are of clinical significance, for example specific LPAR4 deletion improved inflammatory cell recruitment in atherosclerotic lesions.[11,12] Similarly, lysoglycerophospholipids have been reported as active mediators in rheumatoid arthritis (RA) with increased LPA and LPAR1 levels in these patients. Moreover, LPAR1 antagonism modulated synovial inflammation and bone and cartilage damage.[13,14] LPARs are implicated in neuropathic pain[15], renal, liver and pulmonary fibrosis and intimal hyperplasia, responsible for atherosclerosis.[16]

Although well studied in other inflammatory diseases, large clinical studies linking immunometabolic factors and the LPC-ATX-LPA axis in human ALF have not been undertaken. There is evidence in mice that LPA could be protective against paracetamol (acetaminophen, APAP) induced liver injury[17] while LPC induced lobular hepatitis in another animal model.[18] In liver biopsies from organs donated after circulatory death (DCD), LPCs were found increased and this was associated with ischaemic liver injury.[19] Moreover, LPCs were associated with early allograft dysfunction post-liver transplant[20] but little is known of the role of lysophospholipids in the acute setting that often leads to liver transplantation.

This present work addresses a gap in our understanding of immunometabolism in ALF. The aim of this research was to (i) investigate the metabolic signature and the immunometabolic role of the LPC-ATX-LPA pathway in order to find a mechanistic explanation for monocyte behaviour in ALF and (ii) identify possible therapeutic target(s) to modulate the systemic immune response in ALF.

Materials and Methods

Study population

The exploratory cohort included patients with ALF recruited at Kings College Hospital in London between December 2012 and July 2015 ("Gut-Liver Axis in Acute and Chronic Liver Failure Syndromes and Transplantation"; London -Westminster Research Ethics Committee No.: 12/LO/1417; IRAS No.: 104301). The validation cohort included patients admitted from May 2013 to December 2021 to King's College Hospital using the same exclusion criteria (pregnancy, disseminated malignancy, pre-existing immunosuppressive states including drugs and human immunodeficiency virus (HIV) infection and chronic granulomatous diseases). Patients were screened and approached for recruitment as part of the "Monocyte and macrophage phenotype and function in sepsis, acute hepatic failure and chronic liver disease" (London-Westminster Research Ethics Committee No.: 12/LO/0167, IRAS No.: 87203) and the "Immunometabolism in Sepsis, Inflammation and Liver Failure Syndromes/IMET" (North West Haydock Research Ethics Committee No.: 19/NW/0750, IRAS No.: 244089) studies within 24 hours of admission. Patients or family consultees in case of lack of capacity provided written informed consent. Clinical data and laboratory parameters were collected, and disease severity and prognostic scores were calculated, including Model of End-stage Liver Disease (MELD), Child Pugh, and Sequential Organ Failure Assessment (SOFA)[21] (see **Tables 1 and 2 in supplementary** materials).

Isolation of plasma and human peripheral blood mononuclear cells

Blood was drawn into lithium heparin Vacutainers (BD, Franklin Lakes, NJ) and peripheral blood mononuclear cells (PBMCs) isolated as per supplementary materials.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

^{1}H NMR spectroscopy was initially used to profile plasma and identify the main metabolite groups perturbed in ALF. Please see supplementary materials.

Untargeted UPLC-MS lipid analysis

An ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS) method was employed using an untargeted method to generate lipid profiles from serum in both positive and negative ionisation modes.[22] Please see supplementary materials for details.

UPLCMS - BIOCRATES p180 assay

We utilised the AbsoluteIDQ® p180 kit (Biocrates Life Sciences, Austria) to quantify 180 known metabolites as previously described[8]. Please see supplementary materials for details.

Cytokine analysis and ELISAs

Plasma cytokines (*IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α*) were measured using *V-PLEX Proinflammatory Panel 1 Human Kit (*Meso-Scale Discovery,Rockville, MD). ATX, PLA1, PLA2, OPN and M30 were quantified with ELISA as per supplementary materials description.

Single-epitope enzymatic immunohistochemistry for detection of ATX-positive cells.

Liver tissue from explants was stained with primary antibody ENPP2 (Abcam # ab77104, UK). Please see supplementary materials for details.

Cell Culture

Isolated PBMCs from both HC and ALF patients were incubated with different lipids (LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0, LPC 18 - Avanti Polar Lipids, Alabaster, AL) and LPAR antagonists H2L518630 (Sigma-Aldrich), Ro 6842262(Bio-techne) or Ki 16425(Sima-Aldrich). Please see supplementary materials for details.

Immunophenotyping

Monocyte phenotype was determined by flow cytometry on PBMCs using the antibodies in **Table 3** (see suppl meterials).

Monocyte total RNA isolation and mRNA sequencing

Please see supplementary materials for details.

Statistical Analysis

Please see supplementary materials for details.

Complete, Transparent, Accurate and Timely account

Please see Supplementary CTAT Table.

Results

As detailed in the Methods section, the exploratory cohort included 34 acute liver disease (7 acute liver injury or ALI and 27 ALF), 40 healthy controls (HC), 13 stable cirrhosis (SC), 50 acute decompensation of cirrhosis (AD). Transplant free survival at 28 and 90 days and 1 year for ALF were 67%, 56% and 52% respectively (**Table1**). The validation cohort consisted of 62 ALF, 31 HC, 41 patients with stable decompensated cirrhosis [according to PREDICT definition[23],(C)], 31 septic patients (according to SEPSIS-3 criteria)[21]. A sub-cohort was used for metabolomics/lipidomics (**Table 2**). Transplant free survival of ALF patients at both 28 and 90 days was 56%.

AD and ALF are metabolically different from HC according to Multivariate analysis of ^{1}H NMR spectroscopic data

In the exploratory cohort, a three component PCA model of acute liver disease and HCs (R^2=0.48; Q^2=0.38), produced from the data obtained from the ^{1}H NMR spectroscopic analysis of plasma, showed a clear separation between HC and ALI/ALF scores in PC2 which explained 29% of the total variance for the model (**Figure 1A&B**). The PCA loading revealed a decrease in lipid resonances (LDL, vLDL and phosphatidylcholine), as well as a decrease in the branched-chain amino acids (BCAAs) isoleucine and valine and increased lactate, formate and the amino acids lysine, glycine, glutamine and the aromatic amino acids (AAAs) tyrosine and phenylalanine (**Figure 1B**) compared to HCs.

The ALF samples that lay outside the Hoteling's ellipse, and that were separated from all other samples in PC1, also revealed the presence in the plasma ^{1}H NMR spectra of drug related compounds (DRCs), such as sedative and anaesthetic agents, being administered to critically ill patients receiving intensive care.

Untargeted UPLC-MS lipidomic analysis showed reduced LPCs in ALF patients

A previous ^{1}H NMR spectroscopy and UPLC-MS study from our group identified LPCs as the main lipid class driving change seen in survivors vs non-survivors in patients with AD.[24] Based on these findings, an untargeted UPLC-MS lipid profiling method[22] was applied to sera from the exploratory cohort of patients (**Figure1C**). Data were acquired in both positive and negative ESI modes and both sets of data were subjected to multivariate statistical analysis.

PCA modelling of HC and ALF-derived MS results revealed a distinct separation between HC and ALF for both the positive (**Figure 1D**, 2 component model, cumulative R^2=0.59; Q^2=0.52) and negative ESI data (**Figure 1E**, 2 component model, cumulative R^2=0.59; Q^2=0.52). Multivariate modelling using OPLS-DA was performed comparing survival at 30 and 90 days and 1-year post admission. All analysis performed on ALF and ALI groups produced invalid models to predict mortality with low or negative Q^2Y values for 30, 90 and 1-year post admission (data not shown).

Following multivariate statistical analysis 34 discriminating features (**Table 4**), selected from S-plots and VIP plots for ALF vs HC, were subjected to tandem mass spectrometry (MS/MS). Furthermore, 70

features with the highest overall intensities were also subjected to MS/MS (**Table 5**). Fragmentation data generated by MS/MS were used to confirm the tentative identifications (IDs) matched to database searches.

In this way the identities of 4 of the discriminating features (LysoPC(14:0) $[M+H]^+$, LysoPC(18:1) $[M+H+H_2O]^+$, LysoPC(16:1) $[M+H+H_2O]^+$) were confirmed. Pairwise comparisons of ALF vs HC show a decrease in LysoPC(14:0), LysoPC(18:0), LysoPC(18:1), LysoPC(18:2), PC(18:0/20:3), PC(18:1/20:5), SM(d16:1/18:1), SM(d16:1/24:1), SM(d18:1/16:0), SM(d18:1/22:1), SM(d18:1/24:0) and TG(18:2/18:2/18:3) and an increase in PC(14:1/18:0), PC(16:1/18:2) and LPA(18:0e/0:0) in ALF patients compared to HC. A decrease in LysoPC(16:0) was common to SC, AD, ALI and ALF compared to HC.

Hierarchical cluster analysis of lipids identified from UPLC-MS data

Lipids were further assessed to determine correlation patterns in ALF. Correlation heatmaps were computed from the identified positive ESI lipid log_{10} transformed data, using Pearson correlation r^2 (colour scale of -0.5-1.0) separately for HC (**Fig 1F**), acute liver disease (**Fig 1G**) and cirrhosis (**Fig 1H**).

Four main clusters of lipids were determined from metabolite-metabolite correlation analysis of the HC group. In the purple cluster 8 triglycerides showed a strong intercorrelation (r^2>0.7). In the blue cluster there was a strong correlation between phosphocholines (r^2>0.6). Furthermore, PC(16:0/18:1), LysoPC(14:0), PC(16:0/18:2) were correlated with PC (18:0/20:3), TG (16:1/16:0/18:1) and TG (16:0/17:1/18:1) (r^2>0.7). Within the green cluster there was a strong correlation between SMs including SM(d16:1/24:1), SM(d16:1/18:1), SM(d18:1/24:1) (r^2>0.6).

Two main clusters were revealed in the correlation heatmap from the acute liver disease (ALI and ALF) groups. In the red cluster 6 triglycerides were highly correlated (r^2>0.8). Also, in the red cluster, SM(d18:1/24:1), SM(d18:2/24:1), SM(d16:1/18:1), SM(d18:1/16:0) were correlated (r^2>0.7). In addition, SM(d16:1/24:1), SM(d18:1/24:0), PC(18:0/20:3) and PC(16:0/20:3) also from the red cluster were correlated (r^2>0.8). 7 Lysophosphatidylcholines in the blue cluster were correlated (r^2>0.8).

Three main clusters were found in the correlation heatmap from the cirrhosis group. Eight triglycerides were correlated (r^2>0.8). Furthermore, 7 lysophosphatidylcholines were correlated (r^2>0.8). In addition, PC(18:0/20:3), SM(d16:1/24:1), SM(d18:1/24:0), SM(d18:1/22:1) and SM(d18:1/16:0) were also highly correlated (r^2>0.8) and SM(d18:1/16:0), SM(d18:1/24:1), SM(d16:1/18:1) and SM(d18:2/24:1) were highly correlated (r^2>0.8) as well.

Overall, metabolite correlations within the LysoPC and SM groups intensified in both cirrhosis and acute liver disease compared to HCs.

LPC 16:0 was highly discriminant between ALF and HC

In order to confirm our preliminary data, targeted metabotyping (Biocrates Absolute IDQp180) was performed on plasma from the validation cohort including 43 patients with ALF, 24 HC, 21 patients with stable decompensated cirrhosis, and 31 with sepsis (as proinflammatory control). Unsupervised MVA using PCA and supervised OPLS-DA identified LPC 16:0 as highly discriminant between ALF and HC

together with glutamine, alanine, and the phosphatidylcholines 34:2, 36:2 and 36:3 (R2X 0.592; R2Y 0.666, Q2 0.613, AUROC 0.969961) (**Fig 2A,B,D**). From enrichment analysis, phospholipid biosynthesis was the principal pathway differentiating ALF from HC (**Fig 2C**) and cirrhosis but not from sepsis (**Suppl Fig 2**). Moreover, LPC 16:0 remained relevant (within the top 10 metabolites identified with VIP) when samples were analysed using SOFA score as Y in a PLS model (R2X 0.645; R2Y 0.677; Q2 0.599; p= 4.17616e-22), highlighting the correlation with the severity of the disease (**Fig 2E**).

Lysophosphatidylcholines are prognostic markers of poor outcome in ALF

Univariate analysis showed a reduction of LPC 16:0, 18:0 and 18:1 in ALF patients compared to HC and cirrhosis (**Fig 3A**). Moreover, patients with poor prognosis (dead or transplanted at 90 days) had the lowest relative amounts of these lipids e.g. for LPC 16:0 and LPC 18:1 $p<0.05$ (**Figure 3B**). Furthermore, the amounts of these LPCs were negatively correlated with MELD score. (**Suppl Fig 1**) Both for ALF and sepsis no difference was found in the quantities of LPCs observed according to the presence of one particular organ failure (namely renal, circulatory and respiratory) (**Suppl Fig 1F**).

ATX and PLA2 were increased in plasma of ALF patients

In order to explore the source of reduced LPC concentrations in plasma, we studied the main enzymes involved in their metabolism. There was an increase in ATX (ALF vs HC $p<0.001$) and its product LPA in ALF compared to both HC and sepsis (ALF vs HC $p<0.01$). PLA1 was also increased in all diseases (cirrhosis, ALF and sepsis), however PLA2 was only increased in ALF vs cirrhosis (ALF vs C), and vs HC ($p<0.0001$) (**Fig 3C**). Taken together these findings suggest a potential role for liver-ATX mediated conversion of LPC into LPA.

Hepatic ATX expression was increased in ALF

In explants from ALF patients of different aetiologies, ATX expression was observed in areas of viable hepatocytes sparing necrotic areas (**Suppl Fig 2**). This suggests a role of hepatocytes in conversion of LPC to LPA.

ALF patients showed a markedly enhanced cytokine response and increased circulatory makers of cell death

Similarly to sepsis, plasma from ALF patients had increased amounts of both pro-inflammatory (IL-6, IL-8, TNFα) and anti-inflammatory cytokines (IL-10) compared to HC and cirrhotic patients (**Fig 4 and table 1**). Interestingly osteopontin (OPN), a highly modified integrin-binding extracellular matrix glycophosphoprotein produced by the cells of the immune system and a mediator of hepatic macrophage infiltration[25], was increased in ALF also compared to sepsis ($p<0.01$), confirming a role in hepatic inflammation. M30, a caspase-cleaved cytokeratin 18 fragment produced during apoptosis, was increased in ALF compared to HC (**Fig 4C**) and directly correlated with the proinflammatory cytokines (**Fig 4B**). The LPCs 16:0 and 18:0 were directly correlated with lymphocyte count, IL-10, IL-6, IL-8 and IL-1β and inversely with monocyte and neutrophil counts (**Suppl Fig 1D**).

CD14⁺ Monocytes in ALF have a pro-restorative profile with increased MerTK and PD-L1 expression

Phenotypic characterization of PBMCs by flow cytometry showed increased expression of MerTK and PD-L1 in ALF CD14+ monocytes compared to HC (**Fig 5 A-B,** complete phenotyping gating strategy is shown in **Suppl Fig 3A**). Both markers were highly expressed in the intermediate $CD14^{+}CD16^{+}$ and classical $CD14^{+}CD16^{-}$ monocyte subsets (**Suppl Fig 3B**). Immune checkpoints expression on NK and CD56+ T cells did not show a statistically significant difference between study groups (**Suppl Fig 4**). Notably, we observed an increasing trend of CTLA4, PD-1 and PD-L1 positive CD56+CD4+T cells (%) in ALF compared to cirrhosis and healthy control (**Suppl Fig 4B**). The percentage of TIGIT expression was increased in ALF CD56+CD8+T and NK cells compared to other groups but again this was not statistically significant.

PBMCs from ALF patients were hypo-responsive to LPS/LPC/LPA co-culture

We next decided to test *in vitro* cytokine production and its modulation following lipid treatment (**Fig 5C**); therefore, PBMCs were cultured for 24h and then stimulated with LPS (100 ng/mL). Cells from ALF patients produced less IL-6 compared to HC ($p<0.05$). Moreover, LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0 and LPC 18:0 were also tested as potential modulators of the immune response. The addition of LPAs/LPCs failed to reverse cytokines levels in ALF-derived PBMCs and did not affect HC cytokine production.

LPA 16:0 increases and restore PD-L1 expression and reduces MerTK, CD163 and CD155 expression in monocytes

We next questioned whether *in vitro* LPA treatment of PBMCs from HC and ALF patients would alter their phenotypic characteristics. Following incubation with LPA 16:0, the percentage of $CD14^{+}CD16^{+}$ and $CD14^{+}CD16^{-}$ cells within the monocyte pool was reduced (**Fig 5D**). Moreover, after culture with LPA 16:0, LPA 18:0 and LPA 18:1, $CD14^{+}$ monocytes showed immunophenotypic changes (**Suppl Fig 5**). LPA 16:0 treatment increased monocyte PD-L1 expression (% of positive cells, $p<0.05$) in ALF samples, that was reduced in untreated conditions compared to HC ($p<0.05$), and reduced monocyte MerTK, CD163 and CD155 expression in ALF (% of positive cells and MFI, $p<0.05$). Interestingly, no similar effects were observed in T cell subsets (CD8, CD4, and Tregs) which showed unaltered expression of immune checkpoints following LPA treatment (**Suppl Fig 5**).

LPARs expression in monocytes was upregulated during ALF and their reduction had poor prognosis

Given the potential lipid-mediated modulation of monocyte function, we next sought to examine whether hepatic and monocyte expression of LPA receptors (LPARs 1-6) was potentially upregulated. Monocytes expression of LPARs in publicly available microarray data sets was therefore explored. Having already demonstrated in alcoholic hepatitis and HBV-related ALF an increase hepatic expression of autotaxin and LPAR6 (Gene Expression Omnibus dataset GDS4389, series GSE28619/30)[8,26], while in liver samples from paracetamol overdose ALF patients there was a trend of increased expression of LPAR2, 3 and 6 (GSE120652).

In vitro stimulation of monocytes with LPS increased LPAR 1, LPAR 3 and reduced LPAR 6 (GSE61298)[27] (**Suppl Fig 6A**). Moreover CD16 -ve monocytes expressed more LPAR1, and LPAR 6, and reduced LPAR 3 compared to CD16+ve monocytes, confirming a different activation according to monocyte subsets (GSE16836)[28] (**Suppl Fig 6B**). Also, LPARs were no differently expressed according to the outcome in PBMCs of patients with an acute flare of Hepatitis B on chronic infection (GSE168049)[29](data not shown) and in monocyte from patients with paracetamol-induced acute liver failure (GSE80751)[30](**Suppl Fig 6C**).

LPAR identification with monocyte stimulation by receptor antagonists

From our genetic research emerged that LPAR 1, 3 and 6 could be involved in the monocyte phenotype changes observed in ALF. To explore which LPAR played a role in the observed effects, fresh PBMCs were stimulated with the antagonists for the 3 most common receptors (LPARs 1-3); currently there are no commercially available LPAR 4-6 antagonists.

A preliminary study was conducted to decide targets and optimal concentrations of the available antangonists. PBMCs from HC were cultured with antagonists for LPAR 1 (**Ro 6842262),** LPAR2 (**H2L5186303)**, and LPAR 1/3 (**Ki 16425**). We observed that CD155, CD163, MerTK, PD-1 and PD-L1 were reduced by the LPAR 1/3 antagonist (at 1uM) compared to LPA 16:0 (30 uM). This suggests a key role of LPAR 1/3 in monocyte phenotype modulation (**Suppl Fig 7**).

We then focused on LPAR 1 and LPAR 1/3 antagonism for the ALF cells stimulation. LPA 16:0 reduced MerTK and CD163 expression, the effect was statistically significant in HC and with a clear trend also evident in ALF (**Fig 6**). Treatment of PBMCs with LPAR 1 and LPAR 1/3 antagonists restored the MerTK and CD163 expression reduced by LPA 16:0 treatment.

Taken together these data suggest that an LPAR1/3 mechanism underpins the effect of LPA on the regulatory phenotype of monocytes and these receptors are a target for immunotherapy in ALF.

RNA sequencing showed potential pathways of interest unique for ALF

Finally, to explore pathways linking monocyte function and lipid metabolism, we performed mRNA sequencing on isolated monocytes from ALF, decompensated cirrhosis (DC) and HC (**Fig 7A**). As shown in the Venn diagram, 206 genes were unique for ALF (**Fig 7B**). *LPAR1,2,5* and *6* expression levels were not different between study groups (**Fig 7C-D**), while LPAR3 and LPAR4 genes were filtered out due to low expression levels (data not shown).

Of note, *MerTK* and *CD163* monocyte transcript levels were increased in ALF compared to control while Major Histocompatibility Complex related genes (HLA) appeared hypo-expressed in ALF, and the antigen processing and presentation pathways were downregulated (**Fig7E**). *HLA-DPA1, HLA-DPB1, HLA-DRA* and *HLA-DMB* were uniquely downregulated in ALF compared to both groups as well as *CD163, IFNAR 2, FKBP5, ADM* were upregulated in ALF only (**Fig 7D and F**). Moreover, *LAIR1* and *LILRB4*, coding for leucocyte inhibitory receptors were upregulated in ALF compared to both HC and

DC. Among the main pathways discriminating between ALF vs HC and vs DC we found genes related to lipid metabolism including fatty acid metabolism and steroid metabolism, in particular *LPCAT2* and *LPCAT3* which were upregulated in ALF.

Ingenuity pathway analysis by Qiagen IPA (**Suppl Fig 8**), showed the possible relationship between LPARs and monocyte markers. Several pathways were putatively identified, and further research is needed to better explain how to safely modulate monocyte function.

Discussion

Our study is the first to demonstrate the upregulation of the LPC-ATX-LPA axis in both peripheral blood and liver tissue in ALF and its link, *via* an LPAR1/3-related mechanism, with the immune phenotype modulation of circulating monocytes.

We report a crucial role of lipid metabolism in ALF. Lysophosphatidylcholines LPC 16:0 and 18:1, which form the most abundant LPC species in plasma, were identified as discriminant in a population of ALF patients when compared to HC following unbiased ^{1}H NMR spectroscopic and LCMS-based analysis of peripheral blood. These lipids were predictive of poor prognosis in the same population (including mortality or liver transplant at 90 days) and the levels were inversely correlated with MELD score. However, no relationship was found with other organ failures e.g., renal, circulatory and respiratory systems.

Inflammation is accepted as the driving mechanism leading to multiorgan failure and death in ALF populations[31], and monocyte and macrophage dysfunction is central to disease pathogenesis and progression.[2] Initial activation of liver-resident macrophages (Kupffer cells) by damage associated molecular patterns (DAMPs) leads to monocyte recruitment and infiltration. Such effects on monocytes may then contribute to local tissue destruction during the propagation phase and does result in the secretion of pro-inflammatory cytokines.[32,33] In parallel anti-inflammatory cytokines (i.e.IL-10) were increased, suggesting the activation of a Compensatory Anti-inflammatory Response syndrome (CARS) in ALF.[2]

In our cohort pro-inflammatory cytokines increased more than sixfold compared to controls (**Fig 4**) and were correlated with markers of cell death (M30). Subsequently, the recruited monocytes matured into macrophages following local reprogramming towards resolution responses to promote tissue integrity.

Intra-hepatic events may also affect circulating monocytes, which show numerous acquired defects in ALF syndromes, with dysfunctional anti-microbial activity and increased susceptibility to sepsis[2]. What contributes to such reprogramming has been a matter of debate and many soluble mediators have been proposed.[34,2]

Here we have shown that such soluble mediators could include lipids in the LPC and LPA families. The LPCs are lipid metabolites of phosphatidylcholine, synthesized by various enzymes including secretory

phospholipases A2 ($sPLA_2$), HDL-associated lecithin-cholesterol acyltransferase in the reverse cholesterol pathway as well as hepatic and endothelial lipase.[35]
LPAs are thought to be bioactive molecules and their function is exerted by extracellular signalling through at least six ($LPAR_{1–6}$) 7-transmembrane G protein-coupled receptors.[36]

There are two major synthetic pathways for LPAs.[36] Lysophospholipids can be converted to LPA via ATX activity or, alternatively, phosphatidic acid is first produced from phospholipids via phospholipase D, or from diacylglycerol through diacylglycerol kinase, and then converted directly to LPA by the actions of either PLA_1 or PLA_2.
Both pathways appear to be activated in ALF since ATX and PLA2 were increased compared to controls. However, ATX has been found in liver explants obtained from ALF patients, confirming the central role of the liver in the dysregulation of the LPC/ATX/LPA axis in ALF. That this is not purely due to systemic inflammation is evidenced by the lack of ATX increases in patients with severe sepsis.

ALF shares similarities with ACLF, in which cholesteryl esters and lysophospholipids have been considered part of a lipid fingerprint[37]. In addition, PBMC transcriptome analysis has revealed metabolic alterations, including the upregulation of the peroxisome proliferator-activated receptor (PPAR) signalling pathway, cholesterol metabolism, sphingolipid metabolism and glycosaminoglycan biosynthesis[38] and this was confirmed by our data on isolated monocytes from ALF patients.

In this ALF cohort, circulating monocytes showed increased PD-L1 and MerTK expression, confirming a pro-restorative phenotype, previously associated with immunoparesis and increased susceptibility to infections.[2,3,5] Moreover, no significant phenotypic differences were found in blood NK and CD56+T cells in ALF, but this could also be attributed to the early time of sampling. In our recent data from a murine model of paracetamol overdose PD-1 expression in T-cell subsets remained unaltered during the first 3 days after the acute insult. However, PD-L1 expression in Tregs and NKT cells was upregulated at 72 hours post paracetamol overdose, during the pro-restorative phase[3].

We demonstrate a switch in monocyte population with a reduction on CD14+ cells with LPA *in vitro*. Moreover, among LPA 16:0, 18:0 and 18:1, the former induced profound phenotypic changes *in vitro*, reducing CCR2, CD163, CD155 and MerTK and increasing PD-L1 expression in both HC and ALF, without affecting the T-cell population.
In order to identify the LPA receptor responsible for such an effect, we examined LPARs expression in monocytes through publicly available microarray data sets. *In vitro*, M1 monocytes have increased LPAR1, LPAR3 and reduced LPAR6, suggesting a proinflammatory role of for the first two receptors. This is complementary to the previous finding of increased ATX and LPAR1 and 6 in liver tissue from patients with alcohol related liver disease and HBV acute liver failure[8] . We then demonstrated that the LPAR1/3 antagonism showed a similar trend on both cells from HC and ALF with reversing the effect of LPA and increasing CD163 and MerTK.

To evaluate if the phenotypical change was consistent with the gene expression, we performed RNA sequencing on isolated monocytes from patients with ALF, decompensated cirrhosis (DC) and HC. *LPAR1, LPAR2, LPAR5* and *LPAR6* expressions were not statistically different between groups.

Thus, we hypothesised that the circulating lipids stimulate the receptors without inducing differential expression of the genes.

Our numbers were small to analyse outcome prediction, however from public datasets we found that in PBMCs from patients with ACLF secondary to hepatitis B infection and monocyte from paracetamol-induced ALF, LPARs were not differently expressed in patients with poor prognosis.

In our cohort, isolated monocyte *MerTK*, *CD163, LAIR1, LILRB4, S100A9* transcript-level expression was increased in ALF compared to control and Major Histocompatibility Complex related genes *(HLA-DPA1*[39]*, HLA-DPB1, HLA-DRA, HLA-DOA* and *HLA-DMB*) appeared hypo-expressed in ALF confirming an immunosuppressive/pro-restorative profile.

S100A9 is a marker of Myeloid-derived suppressor cells (MDSCs), a population of cells that can negatively regulate T-cell function[40]. Their role has been studied in cancer[41] and by our group in ACLF[42], in which MDSC displayed immunosuppressive properties, decreasing T cell proliferation and reducing bacterial uptake.

Other genes were upregulated only in ALF including ADM (adrenomedullin, a potent vasodilator peptide [44]), IFNAR2 (Interferon Alpha and Beta Receptor Subunit) and FKBP5. The latter encodes for FKBP51 that acts as a negative transcriptional regulator of glucocorticoid receptor and a positive regulator of PPAR signalling. This gene is also related to stress and suicidal behaviour [43], so it will be interesting further exploring it in the setting of acute liver failure and immune dysfunction from paracetamol overdose.

LPS and IFN-γ increase adrenomedullin production by macrophage *in vitro*[45]. Adrenomedullin strongly inhibits LPC–induced migration of human coronary artery smooth muscle cells in a concentration-dependent manner[46]. Its plasmatic concentration has been correlated with vasopressor requirements, organ dysfunction, and mortality in sepsis[47]. Moreover, in decompensated cirrhosis pro-adrenomedullin is associated with short-term survival and ACLF development[48] but its role in ALF is unknown.

Among the main pathways discriminating between ALF vs HC and vs DC we found also genes related to lipid metabolism including fatty acid metabolism and steroid metabolism. Lysophosphatidylcholine acyltransferase (LPCAT) 2 and 3 genes would be of future interest since they are upregulated in ALF and related to the metabolomic signature we discovered. LPCAT2 has been shown to induce macrophage cytokine gene expression and release in response to TLR4 and TLR2[49] and LPCAT3 seems crucial in arachidonic acid reacylation pathway[50] and ferroptosis[51], a novel pathway of iron-dependent necrotic cell death characterized by the accumulation of lipid peroxides. Among the various reactive oxygen species (ROS), lipid peroxides are direct inducers of ferroptotic cell death[52]. One of the crucial steps of this pathway is indeed performed by LPCAT3[53], selectively activated by Interferon-gamma, which incorporates polyunsaturated fatty acids (PUFA)-CoA into phospholipids, using

phosphatidylcholines and phosphatidylethanolamine as substrates. Indeed, the links between lipid metabolism and immune system are multiple and many lipid mediators have been studied in ferroptosis including specialized pro-resolving mediators that would be an interesting research focus in acute liver failure[54].

The aim of the present study was to find potential targets for immunomodulation in acute liver failure. Although ATX has been explored as a target for the treatment of patients with idiopathic pulmonary fibrosis,[55] the heterogenic distribution and expression profiles of LPARs are such, that blocking LPA production globally by inhibiting ATX could lead to severe adverse side effects. Thus, drug development needs to focus on the discovery of novel, potent LPAR ligands targeting a specific receptor subtype without negating the physiological roles of other subtypes[10]. Currently, no drugs targeting LPARs have been approved by the FDA, while several clinical trials are testing LPAR1 antagonists for idiopathic pulmonary fibrosis and systemic sclerosis, and a radioligand that targets the LPAR1 receptor has entered in a phase I clinical trial for positron emission tomography (PET) imaging of idiopathic pulmonary fibrosis.[10]

Our research has several limitations. Total LPA only was measured by ELISA rather than individually. The reason is that LPAs measurement *via* LCMS techniques is challenging. LPAs can be generated *ex vivo* after sample collection and is affected by several chemical and biological processes, making its accurate measurement in biological fluids at risk of confounding[56]. We are currently optimising a technique to efficiently measure LPAs to overcome this limitation. Moreover, we did not measure free and total LPA/LPC, thus the role of albumin binding was not explored in this study.
We used LPS as an immunogenic stimulant and thus only Toll-like receptor 4 (TLR4) mediated inflammation was explored. Other pattern recognition receptors (PRRs) may also be involved and we could therefore have missed some alternative activation pathways.
We studied LPAR antagonism *in vitro* only, thus the multiple effects of LPAR antagonism needs to be elucidated on animal models before applying these findings to ALF patients. Currently only LPAR 1-3 antagonists are commercially available so more information could be gathered once it is possible for LPAR 4-6 antagonism to be explored.

Conclusion: In patients with ALF, lysophosphatidylcholines are reduced and can be used as biomarkers of poor prognosis. The LPC-ATX-LPA axis modulates innate immune response in ALF through LPAR 1 and 3. LPA reduces the pro-restorative phenotypical markers MerTK and CD163 on monocytes and this can be reversed with LPARs antagonism. RNA sequencing unveiled further pathways linking lipid metabolism and immune dysfunction in ALF, distinct from LPARs expression. These preliminary findings highlight the importance of LPARs as possible therapeutic targets in ALF.

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Figure Legend

Figure 1

A&B) **Multivariate analysis of 1H NMR spectroscopic data: ALI and ALF are metabolically different from HC**

In the exploratory cohort, a three component PCA model of acute liver disease and HCs (R2=0.48; Q2=0.38), showed a clear separation between HC and ALF scores in PC2 which explains 29% of total variance for the model. The PCA loading revealed a decrease in lipid resonances (LDL, vLDL and phosphatidylcholine), as well as, a decrease in the branched chain amino acids (BCAAs) isoleucine and valine and raised amounts of lactate and formate and the amino acids lysine, glycine, glutamine and the aromatic amino acids (AAAs) tyrosine and phenylalanine (B) comparing to HCs.

C) Base Peak Ion (BPI) chromatograms of QC serum in ESI+ and ESI- ionisation modes

Abbreviations: FFA - free fatty acids; PC - phosphatidylcholine; PG - phosphatidylglycerol; PE - phosphatidylethanolamine; PI - phosphatidylinositol; PS - phosphatidylserine; SM -sphingomyelin; DG – diacylglycerol.

E&D) PCA modelling of HC and ALF for both positive and negative ESI data, revealed a distinct separation between HC and ALF for both positive (Figure 1D, 2 component model, cumulative R2=0.59; Q2=0.52) and negative mode data (Figure 1E, 2 component model, cumulative R2=0.59; Q2=0.52).

F,G,H) Correlation heatmaps from the identified positive ESI lipid

The lipids detected in positive ESI were further assessed to determine correlation patterns in acute (ALI and ALF) liver disease, as performed previously for the ^{1}H NMR spectral data and the UPLC-MS-detected amines. Correlation heatmaps were computed from the identified positive ESI lipid log10 transformed data, using Pearson correlation r2 colour scale of -0.5-1.0, separately for HC (F), acute liver disease (G) and cirrhosis (H).

Figure 2

A&B) Validation Cohort 180- metabolites panel (Biocrates) confirmed the discriminating power of LPCs

In order to confirm our preliminary data, targeted metabotyping was performed on plasma from the validation cohort including 43 patients with acute liver failure (ALF), 24 healthy control (HC), 21 patients with cirrhosis, and 31 with sepsis (as proinflammatory control). Principal component analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA), identified LPC 16:0 as highly discriminant between ALF and HC together with glutamine, alanine, phosphatidylcholine 34:2, 36:2, 36:3 (R2X 0.592; R2Y 0.666, Q2 0.613, AUROC 0.969961).

C) Enrichment analysis showed phospholipids biosynthesis as the principal pathway differentiating ALF from HC

D) S-Plot confirmed LPC 16:0 as highly discriminant between HC and ALF.

E) LPC 16:0 is related to the severity of ALF

When samples were analysed using SOFA score as Y in a PLS model, LPC 16:0 remained in the top 10 metabolites identified with Variable importance in projection (**VIP**), highlighting the correlation with the severity of the disease

Figure 3

A) LPC is significantly reduced in ALF compared to HC and C but not to sepsis

Kruskall-Wallis test HC n=24,C n=21, ALF n=43, Sepsis n=31. HC vs ALF **** p< 0.0001

Graphs show mean +SEM

B) LPC 16:0, LPC 18:0 and LPC 18:1 were reduced in patients with poor prognosis (dead or transplanted at 90 days post admission).

Alive n= 24, dead/transplanted n=19

Mann-Whitney test: LPC 16:0, *p=0.0339; LPC 18:0 p=0.06; LPC 18:1 *p=0.0434

Graphs show mean +SEM

C) Autotaxin and PLA 2 are significantly increased in ALF.

ATX: HC n=31, C=41, ALF= 62, Sepsis=31, Kruskal Wallis, HC vs ALF **** p< 0.0001

LPA: HC= 14, C=38, ALF=18, Sepsis=27, Kruskal Wallis, HC vs ALF ** p=0.0022

PLA 1: HC n=16, C n=16, ALF n=32, Sepsis n=24, one way ANOVA, HC vs ALF *** p= 0.0006

PLA 2: HC n=10, ALF n= 40, n=20, sepsis n=24, Kruskal Wallis, HC vs ALF **** p< 0.0001

Graphs show mean +SEM. * p< .05, ** p< .01, *** p< .001, **** p< .0001

Figure 4

Increased inflammatory cytokine plasma levels correlate with LPC-ATX-LPA dysregulation in acute liver failure

A) ALF patients had increased plasma proinflammatory cytokines (validation cohort). HC n=18, C n=30, ALF n=20, sepsis n=16. Graphs show mean + SEM. Kruskall-Wallis.

Most relevant comparisons are shown in detail:

HC vs ALF: GMCSF **** p< 0.0001, IFN gamma p>0.9999, IL-10 **** p< 0.0001, IL1B p>0.9999, IL2 p=0.7178, IL6 **** p< 0.0001, IL8 **** p< 0.0001, TNFa **** p< 0.0001, Osteopontin (OPN) **** p< 0.0001

B) Correlations Matrix: M30 is directly correlated with proinflammatory cytokines in particular with TNF alpha. Soluble Mer does not correlate with other immunological markers. Correlation matrix, Spearman's Correlation, correlation coefficients (rho) are showed per each pair in the corresponding cell.

C) M30, a caspase-cleaved **cytokeratin 18 fragment** produced during apoptosis, was increased in ALF compared to HC. Graphs show mean +SD, Kruskall-Wallis test, HC vs ALF ***p=0.0006

* p< .05, ** p< .01, *** p< .001, **** p< .0001

Figure 5

Monocyte in ALF show a prorestorative phenotype

A) Gating strategy, markers including MerTK were assessed on live CD14+ cells.

B) MerTK and PD-L1 expression is increased in fresh CD14+ cells in ALF(n=7) compared to HC(n=8). Most relevant comparisons are shown in detail:
Mann-Whitney test, PD1% p=0.955, PD1 MFI *p=0.0306, PD-L1% **p=0.0059, PD-L1 MFI *p=0.0434, MerTk % *p=0.0140, MerTk MFI p=0.2319
Graphs show mean +SEM, * p< .05, ** p< .01, *** p< .001, **** p< .0001

C) PBMCs from ALF patients produced less IL-6 when stimulated with LPS (p<0.05). Moreover, LPA 16:0, LPA 18:0, LPA 18:1, LPC 16:0 and LPC 18:0 were tested as potential modulators of immune response. The addition of LPAs/LPCs failed to reverse the immune dysfunction in ALF PBMCs and did not affect HC function. Mixed Effects analysis, Most relevant comparisons are shown in detail:
HC vs ALF: LPS only *p=0.0135, LPA 16:0 *p=0.0309. The other comparisons were not statistically significant with p>0.05
Graphs show mean +SEM, * p< .05, ** p< .01, *** p< .001, **** p< .0001

D) **LPA modifies monocyte phenotype**
Percentage of CD14+ cells decreased after 24h PBMCs co-culture with LPA 16:0
Most relevant comparisons are shown in detail:
2way ANOVA, ALF LPA vs blank, CD14-CD16+ p= 0.9907, CD14+CD16+ *p=0.0125, CD14+CD16- p=0.1336
2way ANOVA, HC LPA vs blank, CD14-CD16+ p= 0.8709, CD14+CD16+ ***p=0.0001, CD14+CD16- p=0.0693
Graphs show mean +SEM, * p< .05, ** p< .01, *** p< .001, **** p< .0001

Figure 6

LPAR 1 and 3 were the key receptors leading to LPA induced monocyte phenotypical changes
In PBMCs culture, LPA 16:0 reduced MerTK and CD163 expression in CD14+ cells. Treatment with LPAR 1 and LPAR 1/3 antagonists, restored MerTK andCD163 expression reduced by LPA 16:0 treatment. Mixed effects analysis. Most relevant comparisons are shown in detail:
HC LPA 16:0 vs Ki 16425, CD163% *p=0.0215, MerTk% *p=0.0276, MerTk MFI *p=0.0197
HC LPA 16:0 vs Ro 6842262, CD163% *p=0.0225, MerTk% *p=0.0266, MerTk MFI *p=0.0442
Graphs show mean +SEM, * p< .05, ** p< .01, *** p< .001, **** p< .0001
MerTK: Mer tyrosine kinase, PD1: Programmed cell death 1, PD-L1:Programmed cell death ligand-1, Ki16: Ki 16425, Ro68: Ro 6842262

Figure 7

RNA sequencing of monocytes unveiled pathways of interest in acute liver failure
A: Principal Component Analysis for RNA sequencing data showing the 3 study groups: acute liver failure (ALF n=4), decompensated cirrhosis (DC n=6) and healthy controls (n=3).
B: A Venn diagram showing the overlap of Differentially Expressed genes between the three different pairwise comparisons using p<0.05 and |logFC|>1.5

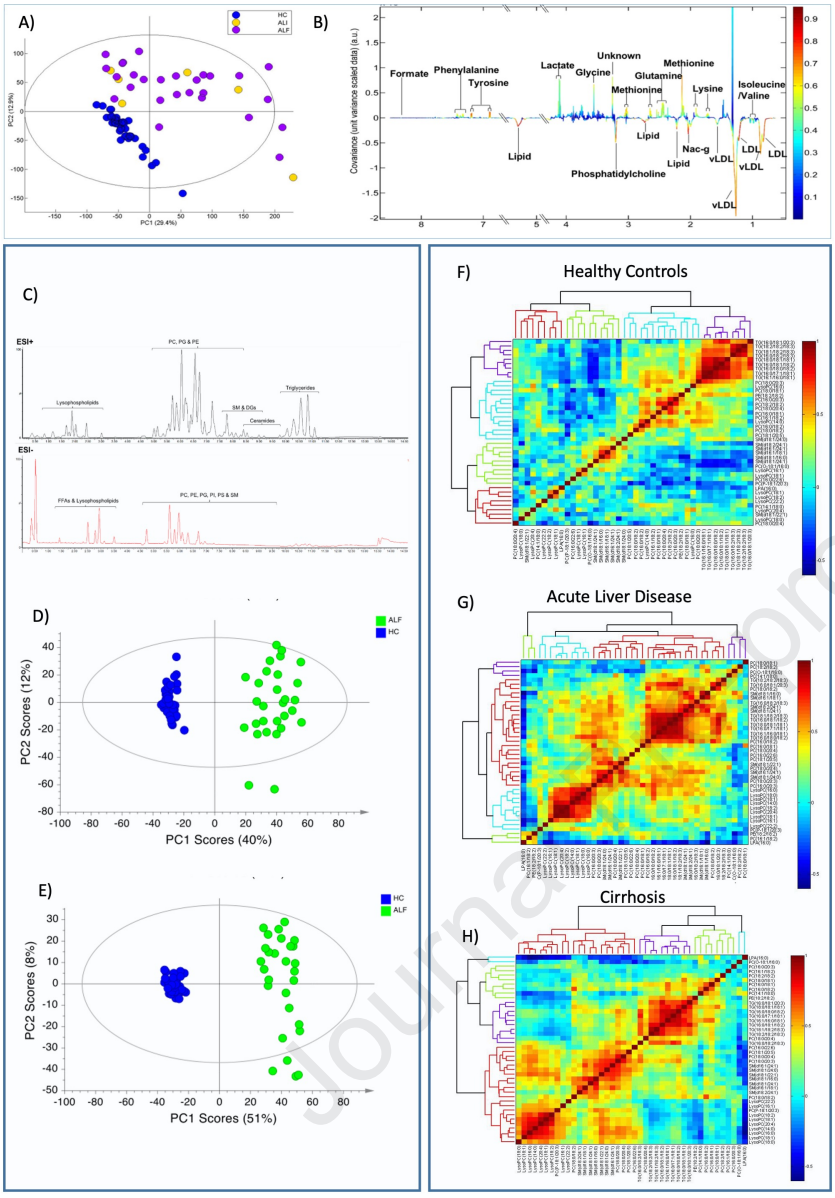
C: Volcano plots showing the differential expression in Log2 fold change of genes between ALF vs HC and ALF vs DC.

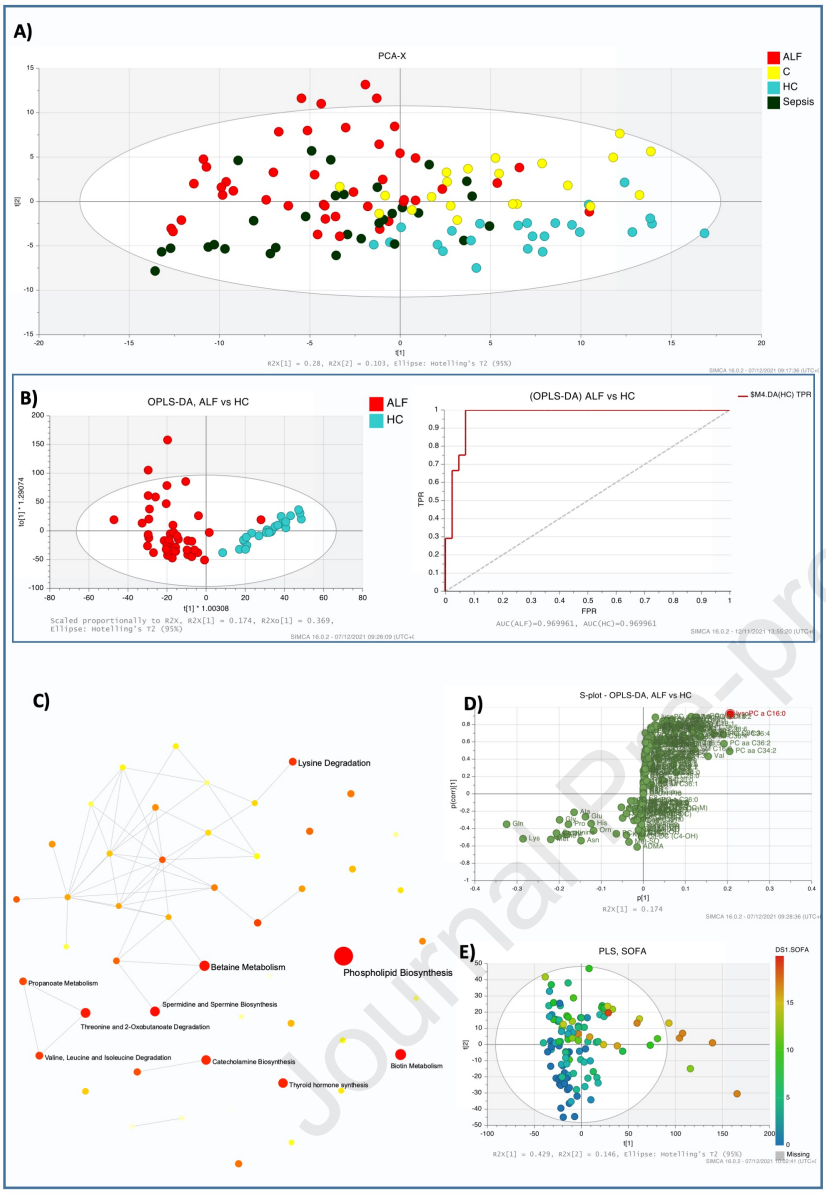
D: Box plots of LPAR genes of interest in Acute liver failure (ALF), Decompensated cirrhosis (DC), Healthy controls, LPAR 3 and 4 were detected but filtered out due to low expression levels.

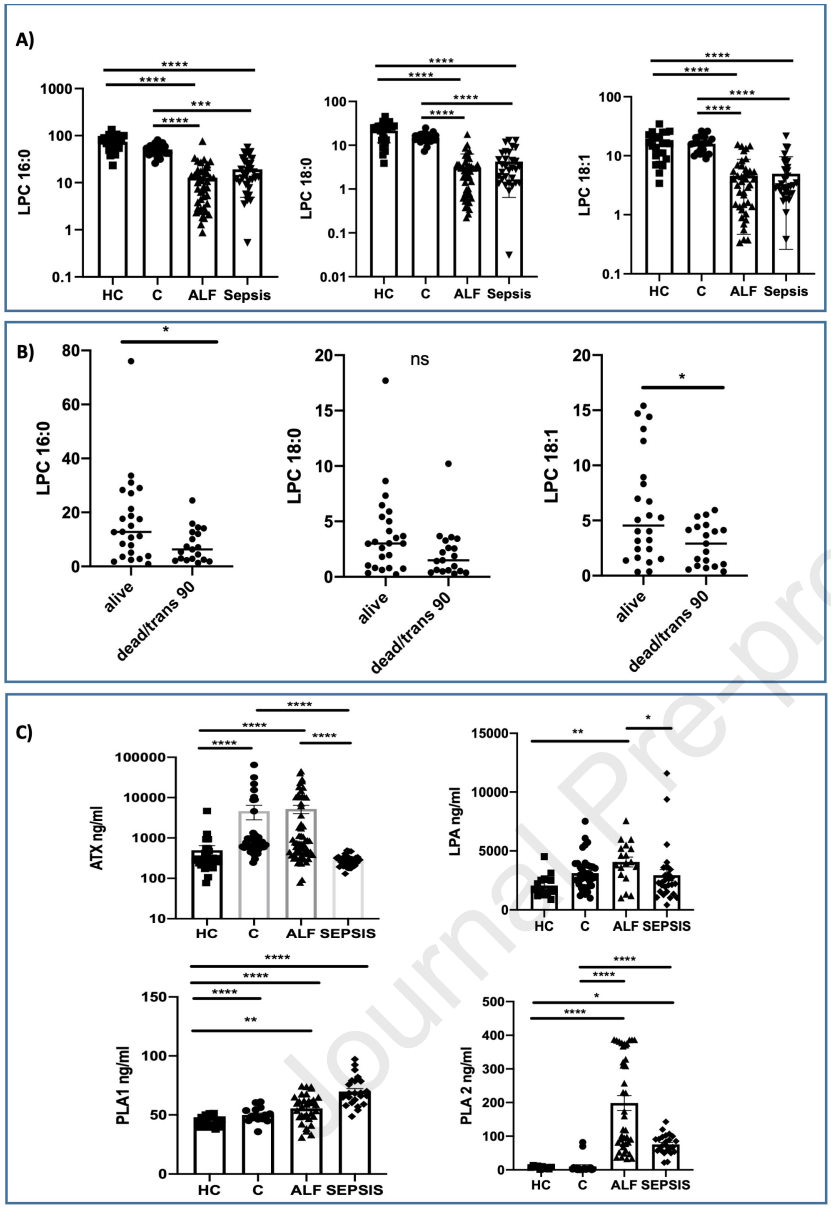
Kruskall-Wallis, not significant. LPAR1 p=0.2250, LPAR2 p=0.5571, LPAR5 p=0.4340, LPAR6 p=0.4058

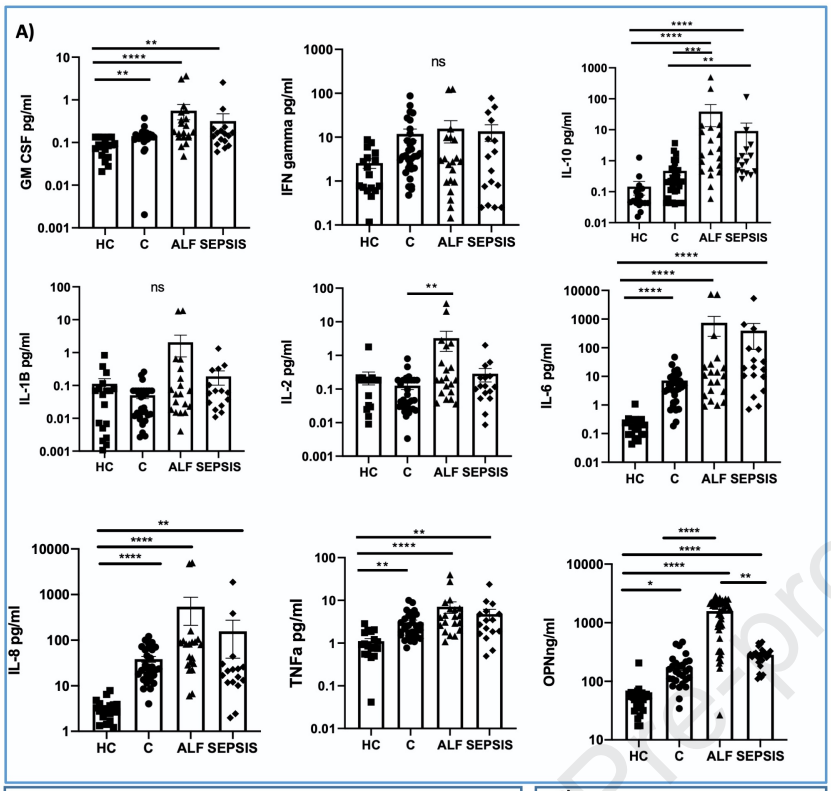
E: Pathways analysis plots based upon the GAGE analysis for the pairwise comparisons ALF vs HC and ALF vs DC.

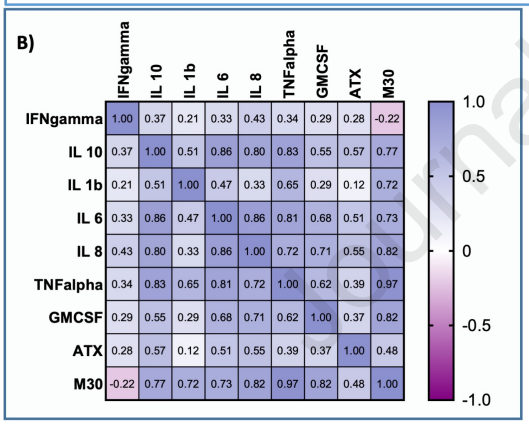
F: Differential expression in Log2 fold change of genes involved in lipid metabolism and immune response

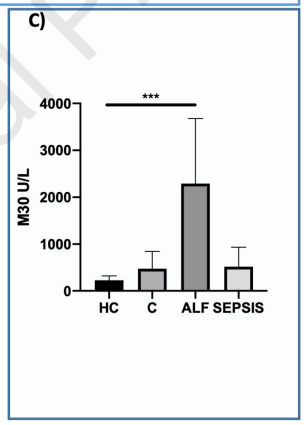


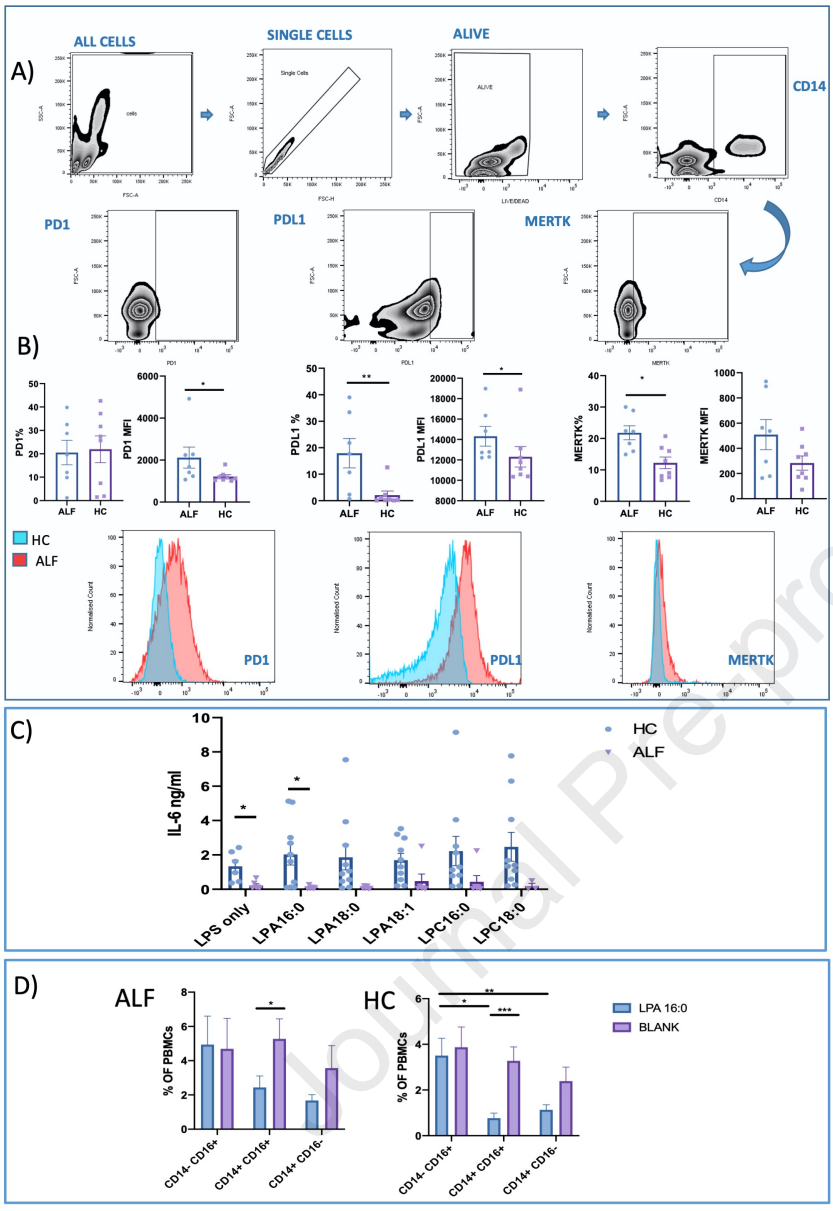


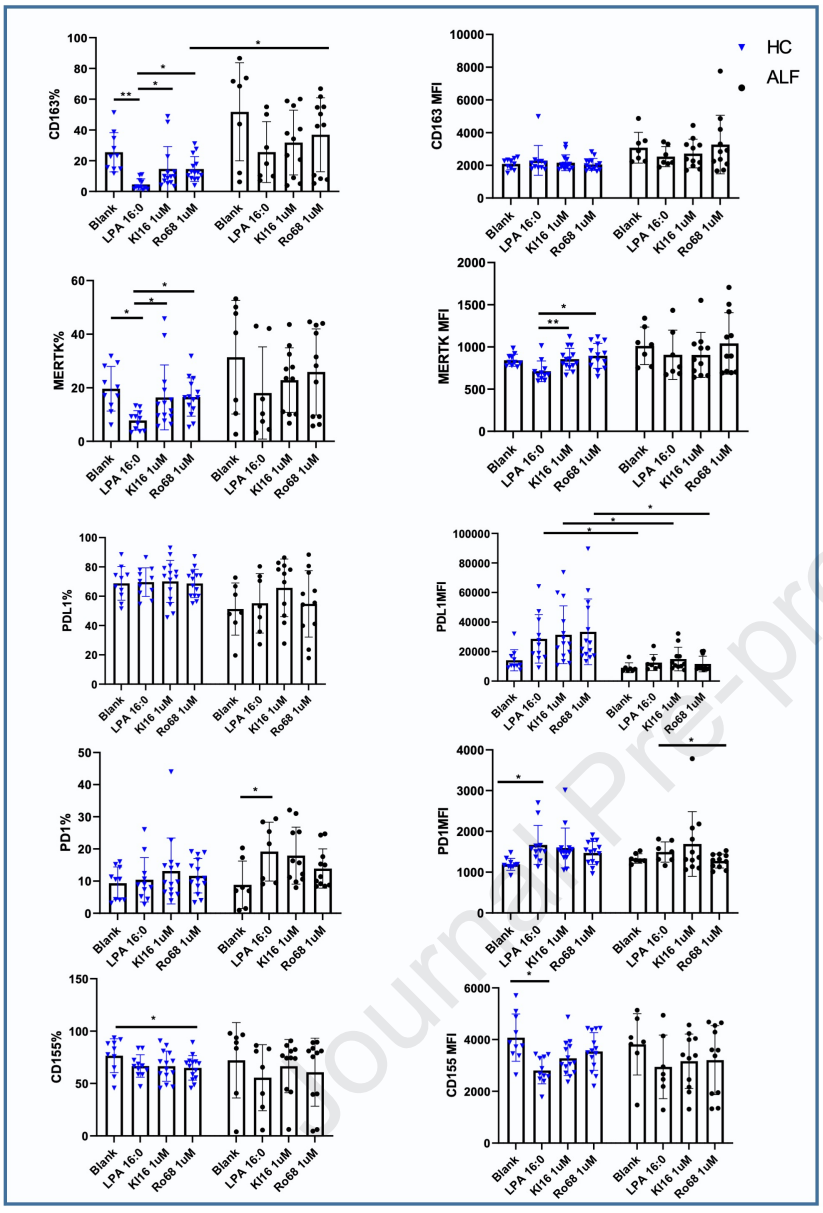


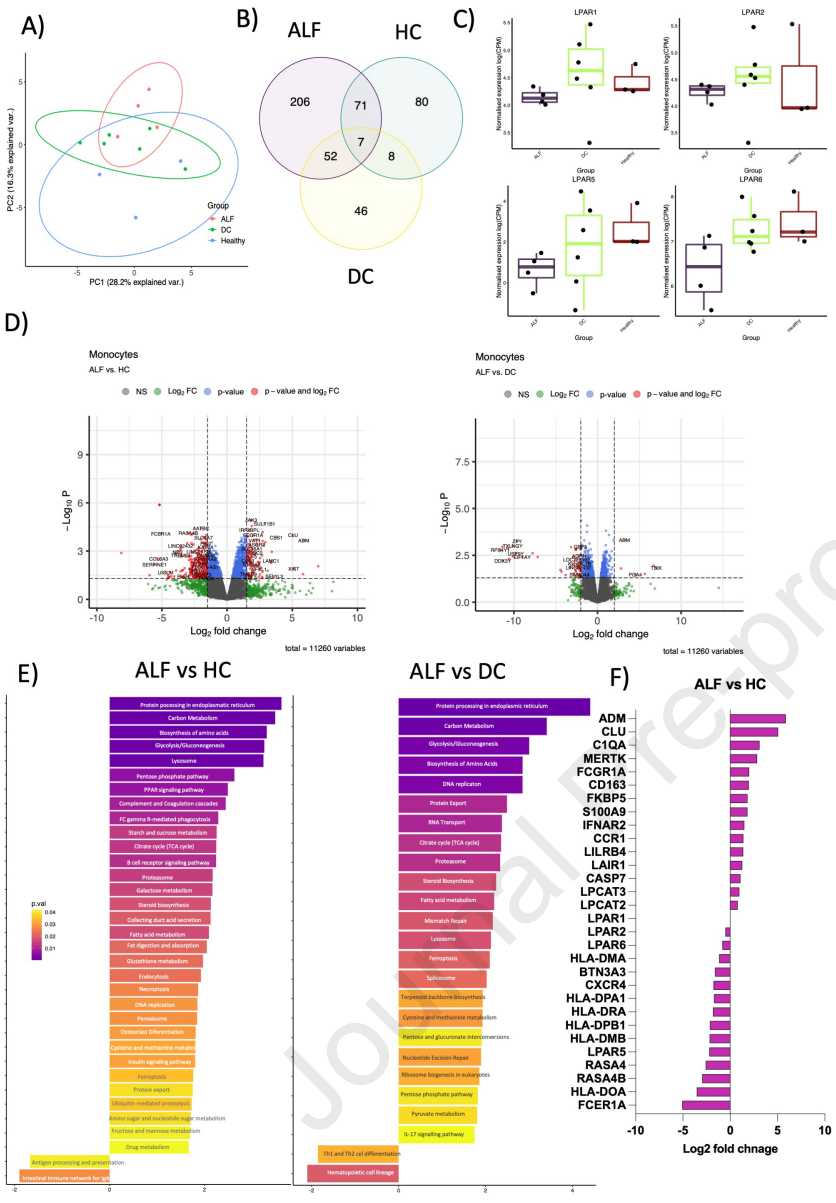

A)
GM CSF pg/ml
IFN gamma pg/ml
IL-10 pg/ml
IL-1B pg/ml
IL-2 pg/ml
IL-6 pg/ml
IL-8 pg/ml
TNFa pg/ml
OPNng/ml
HC
C
ALF SEPSIS
ns


B)
IFNgamma
IL 10
IL 1b
IL 6
IL 8
TNFalpha
GMCSF
ATX
M30
1.00 0.37 0.21 0.33 0.43 0.34 0.29 0.28 -0.22
0.37 1.00 0.51 0.86 0.80 0.83 0.55 0.57 0.77
0.21 0.51 1.00 0.47 0.33 0.65 0.29 0.12 0.72
0.33 0.86 0.47 1.00 0.86 0.81 0.68 0.51 0.73
0.43 0.80 0.33 0.86 1.00 0.72 0.71 0.55 0.82
0.34 0.83 0.65 0.81 0.72 1.00 0.62 0.39 0.97
0.29 0.55 0.29 0.68 0.71 0.62 1.00 0.37 0.82
0.28 0.57 0.12 0.51 0.55 0.39 0.37 1.00 0.48
-0.22 0.77 0.72 0.73 0.82 0.97 0.82 0.48 1.00
1.0
0.5
0
-0.5
-1.0


C)
M30 U/L
HC
C
ALF SEPSIS







Lysophosphatidylcholines are associated with mortality in acute liver failure and modulate immunoregulatory checkpoints in peripheral monocytes

Francesca M Trovato et al.

Highlights

- *Plasma lysophosphatidylcholines are reduced and can be used as prognostic markers of poor outcome in ALF*
- *The LPC-ATX-LPA axis appears to modulate innate immune response in ALF via MerTK and CD163 expression*
- *LPAR1 and LPAR3 antagonism reverses the effects of LPA on monocyte phenotype*