pectively; $p < 0.0001$). Concerning patients treated with
nids, CLIF-OF at 3–7 days after diagnosis, was not a
tly better predictor of 28- and 90-day mortality than Lille
ROC [95% CI] 0.95 [0.86–1.00] vs 0.95 [0.85–1.00] and 0.96
)] vs 0.92 [0.84–1.00], respectively).
ns: AH was associated with a high short- and mid-term
rate. Assessment of CLIF-OF at 3–7 days results in a more
prediction of 28 and 90-day mortality than at admission.
may be useful predictor of mortality especially in patients
d with corticosteroids.

**HANISMS UNDERLYING MURINE AND HUMAN
EN INTOXICATION**

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nd and Aims: Ibuprofen is a commonly used non-steroidal
nmatory (NSAID) drug, available both by prescription and
counter. Although it is generally well-tolerated, Ibuprofen
e serious liver injury. Ibuprofen intoxication ranges from
in serum aminotransferase levels to acute liver failure
ven the need for liver transplantation. Thus, we aimed to
e molecular pathways by which ibuprofen induces ALF
present, remain elusive.

We studied clinicopathologic characteristics of patients
uprofen intoxication (>0.1–2 grams). Moreover, we
ed the activation of essential pathways in Ibuprofen-
ug induced liver injury (DILI) in liver sections. In parallel,
mined Ibuprofen cytotoxicity by calculating the median
e (LD50) in Hepa 1-6 cells and applied it to cultures of
epatocytes isolated from C57BL/6 mice. Next, we translated
s of the *in vitro* cytotoxicity into a murine model of
intoxication. Overnight fasted male C57BL/6 mice (6–8
age) were i.p injected with either DMSO or 600 mg/kg

JNK phosphorylation was evident in the cytoplasm of
es in Ibuprofen-induced ALF liver samples compared
hy tissue. The calculated LD50 was 7.5 mM after 8 h of
with Ibuprofen. Primary murine hepatocytes cultured
profen evidenced increased cell death (TUNEL),
ory proliferation (Ki-67), production of reactive oxygen
2-DCFDA) and mitochondrial dysfunction (Mitosox).
ion of Ibuprofen produced a greater level of liver injury
observed in DMSO-treated control mice, based on levels of
kers and microscopic and histologic analysis of liver
ext, we sought to investigate the molecular pathways
with Ibuprofen overdose in mice. Interestingly we found
ctivation of PKCα, AKT and JNK, and decreased p38 protein
hours after Ibuprofen challenge.
ns: In our study, we first studied Ibuprofen cytotoxicity in a
primary hepatocytes, which we translated into a murine
buprofen intoxication. While cytoplasmic JNK activation
mark of Ibuprofen intoxication in human samples,
ctivation of the PKCα/AKT/JNK pathway together with
of the p38-signaling cascade were features of murine
toxication. These results open novel clinical targets for
ent of Ibuprofen-ALF patients.

THU-304

NOVEL FUNCTION OF MITOCHONDRIAL LON PROTEASE (LONP) IN A DRUG-INDUCED DUAL MODEL OF ER-STRESS AND MITOCHONDRIAL DYSFUNCTION IN HEPATIC CELLS

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Background and Aims: Mitochondrial ATP-dependent protease Lon (LONP) supports cell viability during mitochondrial, oxidative and endoplasmic reticulum (ER)-stress. The non-nucleoside analog reverse transcriptase inhibitor Efavirenz (EFV), one of the most widely employed anti-HIV drugs, has been associated with hepatic toxicity linked to a mitochondrial effect accompanied by ER-stress/unfolded protein response (UPR). We investigatedthe abundance of mitochondria-associated ER membranes (MAMs) and LONP in an in vitro model of EFV exposure.
Methods: Human hepatoma cell line Hep3B was treated (24 h) with clinically relevant concentrations of EFV, comparing it with other mitochondrial stressors: Complex I inhibitor rotenone, ER-stress inductor thapsigargin and the uncoupler CCCP. Cell biology techniques (Western blot or RT-PCR) were used to analyze expression and localization of LONP and the main regulators of the mitochondrial dynamics. Co-immunoprecipitation and confocal fluorescence microscopy were employed to study MAMs.
Results: EFV enhances the presence of MAMs and alters mitochondrial dynamics. It induces mitochondrial fission and decreases fusion, detected by upregulated expression of Fis1 (Mitochondrial Fission 1 Protein), Mff (Mitochondrial Fission Factor) and Drp1 (Dynamin-Related Protein 1). A greater translocation of active Drp1 to mitochondria was observed (confocal microscopy). Markers of altered fusion were also evaluated; Optic Atrophy 1 Protein (OPA1) was induced together with its proteolysis and Mitofusin 2 (Mfn2) gene expression was augmented. MAMs were analyzed by assessing GRP75 (Heat Shock 70 kDa Protein 9), Sigma1-Receptor (Sig-1R) and the interaction of VAPB/C (Vesicle-Associated Membrane Protein-Associated Protein B/C) with PTPIP51 (Protein Tyrosine Phosphatase-Interacting Protein 51) which was enhanced. Notably, both gene and protein levels of LONP were increased as was surprisingly, its presence in the cytosol. EFV response revealed both similarities and differences with other mitotoxic agents confirming the specificity of a combined ER/mitochondrial stress.
Conclusions: Specific dual mitochondria-ER effect as that triggered by the antiretroviral drug EFV enhanced MAMs content in hepatic cells, which was associated with increased extra-mitochondrial LONP expression. This is the first report of this phenomenon in mammalian cells and it may play a significant role in the hepatic adverse events related to the clinical use of EFV.

THU-305

N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND PROGNOSIS OF ALCOHOLIC LIVER CIRRHOSIS COURSE

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Background and Aims: The brain natriuretic peptide (BNP) and its N-terminal prohormone (NT-proBNP) are sensitive markers of cardiac dysfunction. The aim of this study was to investigate the prognostic role of NT-proBNP of cardiac and renal dysfunction and liver disease severity in alcoholic liver cirrhosis (ALC) patients.
Methods: Forty-two patients with ALC in various stages II and III [median age, 59.5 yr, 21.4% females] without history of previous cardiac/ or renal disease were divided into 3 groups according to the Child-Pugh classification: grade A (n = 12), B (n = 15), and C (n = 15). ALC diagnosed by clinical, biochemical and ultrasonographic

findings; cardiac dysfunction – by clinical, electrocardiography and echocardiography findings; renal dysfunction – by glomerular filtration rates (GFR), serum creatinine levels and micro-/macroalbuminuria. Circulating NT-proBNP levels were measured by the immuno-assay method. ALC patients were monitored for 2 years. 12 healthy volunteers [median age, 59.2 yr, 25.0% females] were the control group.

Results: The median NT-proBNP serum levels were significantly increased in patients with decompensated ALC (grade B: 926.5 pg/mL, grade C: 1450.3 pg/mL versus grade A: 339.4 pg/mL) compared control group (66.2 pg/mL) ($p < 0.001$). The NT-proBNP levels correlated with the Child-Pugh score ($r = 0.56$, $p < 0.001$), bilirubin levels ($r = 0.48$, $p < 0.01$) and albumin levels ($r = -0.39$, $p < 0.01$). The high NT-proBNP levels in ALC patients were associated with cardiac dysfunction by reduced left ventricular (LV) ejection fraction and increased LV mass ($r = -0.41$, $p < 0.01$; $r = 0.51$, $p < 0.001$) and renal dysfunction by decreased GFR and albuminuria ($r = -0.45$, $p < 0.01$; $r = 0.36$, $p < 0.03$). In following 2 years death occurred in 2 (13.3%) of ALC patients grade B and in 7 (46.7%) of ALC patients grade C of Child-Pugh. NT-proBNP ≥1000 pg/mL was associated with an increased risk of death over 2 years in decompensated ALC patients (adjusted HR 2.49 [95% CI 1.25–5.88], $p = 0.03$).

Conclusions: The high NT-proBNP levels in patients with decompensated ALC may be an independent predictor of cardiac and renal dysfunction as well as severe ALC course and increased risk of death over 2 years.

THU-306
HIGH FREQUENCY OF INFLAMMATORY CD16+ MONOCYTES IN ALCOHOLIC HEPATITIS CAN BE REDUCED BY TREATMENT WITH PREDNISOLONE

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Background and Aims: Severe alcoholic hepatitis (SAH) is an inflammatory condition associated with the systemic inflammatory response syndrome and high serum levels of inflammatory cytokines. Accordingly, 28-day mortality in this condition can be reduced by treatment with the anti-inflammatory corticosteroid prednisolone. We sought to evaluate the impact of prednisolone versus placebo on the phenotype and function of circulating monocyte subsets in SAH.

Methods: We sampled blood from 23 patients with SAH (DF > 32) participating in the STOPAH study; 34 healthy controls (HC); and 9 patients with cirrhotic compensated alcoholic liver disease (CLD). Monoclonal antibody and FACS was used to identify monocyte subsets (classical $CD14^+CD16^-$, intermediate $CD14^+CD16^+$ and non-classical $CD14^{lo}CD16^+$) and surface activation and chemokine receptor markers. Intracellular cytokine staining was used to quantify monocyte subset responses to 100 ng/mL lipopolysaccharide (LPS).

Results: The population of intermediate monocytes was expanded in SAH (11% vs 6% HC $p < 0.001$), and the frequency of patrolling non-classical monocytes was conversely diminished (1% vs 5% HC; $p < 0.0001$). Intermediate monocytes expressed higher levels of the activation marker HLA-DR (vs CLD; $p = 0.005$). Accordingly, median production of Il-1β, IL-6 and IFN-γ in response to LPS was higher in intermediate compared to classical monocytes, and significantly for TNF-α ($p = 0.03$). These intermediate monocytes also bore higher expression of the chemokine receptor CCR-5 (vs HC; $p = 0.01$). Strikingly, the frequency of intermediate monocytes was reduced after 7 days treatment with prednisolone (15% reduced to 6%; $p = 0.05$) vs patients who were treated without prednisolone (11% to 10%; $p = 0.9$). 7 days treatment with prednisolone also reduced expression of the activation marker HLA-DR on intermediate monocytes

without predni
HLA-DR after 7

Conclusions: Th
monocyte subset
novel mechanism
prednisolone in
high expression
subset opens av
inflammation with
prednisolone thera

THU-307
NATURAL KILLER
CELLS IN ALCOHOL
INCREASING PRO-I

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Background and A
alcoholic liver diseas
cells (NK), avital com
hepatocellular damag
aim to investigate the
of endothelial progen

Methods: Patients wi
etiologies (CLD crypto
were recruited under
by flow cytometry and
$CD31^{lo}CD105 + VEGRF-$
and NK cells (CD3- CD
specific EPCs were cul
byLDL uptake and sp
modulate EPCs was che
line and ex vivo with
culture experiments w
were also testedin an e
steatosis using mouse s

Results: Two distinct
($CD45^{hi}$ $CD31^{lo}$) and late
blood. The frequency of
controls ($p = 0.007$, $p = 0$.
markers, VEGRF2 and C
derived from ALD con
$CD31^{hi}CD34 + CD133 + VI$
$CD31^{hi}CD34 + CD133 + VI$
the blood and liver of mic
compared to saline cont
Frequencies of NK and NI
patients ($p = 0.02$, $p = 0.0$

Alcoholic liver disease and drug induced liver disease (cont.)

THU-301 YI
MCJ/DNAJC15, THE MITOCHONDRIAL FOE IN LIVER INJURY
Lucia Barbier Torres*, Paula Iruzubieta, Daniel Taibo, Teresa Cardoso, Nicolas Navasa, David Fernández Ramos, Marta Varela Rey, Virginia Gutiérrez de Juan, Pablo Fernández Tussy, Imanol Zubiete Franco, Maria Isabel Hernández Alvarez, Raul Andrade, Inmaculada Medina, María Jesús Monte, José Juan García Marín, Javier Crespo, Antonio Zorzano, José María Mato, Juan Anguita, Mercedes Rincon, María Luz Martínez Chantar, *Spain*

THU-302
PREDICTING MORTALITY IN ALCOHOLIC HEPATITIS USING CLIF-ORGAN FAILURE SCORE
Marco Silva*, Patricia Andrade, Susana Rodrigues, Armando Peixoto, Rui Gaspar, Susana Lopes, Hélder Cardoso, Guilherme Macedo, *Portugal*

THU-303 YI
THE MECHANISMS UNDERLYING MURINE AND HUMAN IBUPROFEN INTOXICATION
Miguel Eugenio Zoubek*, Marius Maximilian Woitok, Raul J. Andrade, M. Isabel Lucena, Christian Trautwein, Francisco Javier Cubero, *Germany*

THU-304
NOVEL FUNCTION OF MITOCHONDRIAL LON PROTEASE (LONP) IN A DRUG-INDUCED DUAL MODEL OF ER-STRESS AND MITOCHONDRIAL DYSFUNCTION IN HEPATIC CELLS
Miriam Polo, Fernando Alegre, Alberto Marti-Rodrigo, Ana Blas-Garcia, Juan V Esplugues, Nadezda Apostolova*, *Spain*

THU-305
N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE AND PROGNOSIS OF ALCOHOLIC LIVER CIRRHOSIS COURSE
Nataliya Virstyuk*, Iryna Kobitovych, Nataliya Slyvka, Oleg Virstyuk, *Ukraine*

THU-306 YI
HIGH FREQUENCY OF INFLAMMATORY CD16+ MONOCYTES IN ALCOHOLIC HEPATITIS CAN BE REDUCED BY TREATMENT WITH PREDNISOLONE
Nikhil Vergis*, Wafa Khamri, Charalambos Antoniades, Mark Thursz, *United Kingdom*